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UPTAKE OF IONS AND METALLIC CHELATION IN PLANTS*

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The essentiality of micronutrients for the normal metabolic functions of plants has been shown by many (Brenchley, 1936; Javillier, 1908; Hewitt, 1951), and there is no lack of experimental evidence to show that an altered nutrition of the host is mainly responsible for their susceptibility or resistance to disease, particularly to attack by root-disease pathogens (Walker and Hooker, 1945; Walker and Foster, 1946; Garrett, 1941, 1956). Although resistance or susceptibility has primarily a genetic basis in plants, their potentiality of becoming resistant or susceptible to an invading pathogen may be largely influenced by environment. The conditions prevailing before and after infection affect the physiology of the host and hence its capacity for excluding or for supporting development of the parasite. Much of the earlier postulates on the physiology of host-parasite interaction have largely been replaced in recent years by Brown's theory of enzyme-substrate interaction (Brown, 1934, 1936, 1955). In the course of a critical analysis of the conditions necessary for the establishment of true parasitism, Garrett (1956) has recently stressed the need for a full appreciation of the relative importance of the following factors: (*i*) the presence of a susceptible host, (*ii*) the presence of a parasite, and (*iii*) the occurrence of suitable environmental conditions for penetration and infection of the host. From a biochemical angle, it may be stated that the logical prerequisite for these conditions would be (*a*) a host substrate of composition suitable for the enzymatic activity of the parasite *in vivo*, (*b*) the presence of suitable enzyme systems in the parasite for the enzyme-substrate interaction during disease, and (*c*) the availability of optimal conditions for successful enzymatic breakdown. The important role of trace elements as bio-catalysts of enzyme action has been widely recognised. Most workers feel that like the vitamins, trace elements perform their important functions in the nutrition of plants by assuming a catalytic function in enzyme systems.

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Earlier work in this laboratory has indicated the role of micronutrients in altering host susceptibility, especially in the fusariose wilts (Sadasivan, 1950, 1951, 1952; Sadasivan and Subramanian, 1954; Sarojini, 1951; Sulochana, 1952; Varadarajan, 1953; Kalyanasundaram, 1954; Satyanarayana, 1955). The exact mechanism through which this is attained is variously described—increase in the antagonistic microflora in the rhizosphere, changes in the carbohydrate and protein metabolism representing unfavourable conditions for both pre-penetration and post-penetration phases of infection. It was also pointed out (Subramanian, 1956 *a*) that the quantum of available free trace elements in the host plants would determine susceptibility or resistance to wilt in cotton, in view of the important role of chelation of phytotoxins with heavy metals *in vivo* and their consequent potentiation. Kalyanasundaram and Saraswathi-Devi (1955) reported that an optimal concentration of zinc was essential for the production of fusaric acid and higher concentrations of the metal were inhibitory for its production and thus the concentration of available zinc in the host would appear to be an important factor which affects the production of this vivotoxin.

Gäumann (1951) has clearly indicated that resistance of the host depends on its ability to check the generalisation of the pathogen rather than its ability to counteract the toxic metabolites. In the resistant varieties the infection remains restricted to the primary focus and the fungus fails to develop and produce sufficient quantum of the toxin. It would be of interest to mention in this connection Lakshminarayanan's (1955) work on pectin distribution in cotton plants which indicated that the resistant roots recorded low pectin levels which resulted in non-secretion or secretion in low amounts of the adaptive pectic enzymes and consequent localization of the parasite. Secretion of suitable exo-enzymes by the pathogen that could attack the host far in advance of the mycelial growth would provide a plausible explanation of the mechanism of their vivotoxic action. The pectic enzyme secretion by plant pathogens like *Fusarium vasinfectum* fits in excellently well with the definition of vivotoxin (Waggoner and Dimond, 1953). Recently very considerable attention has been directed to the study of the role of these enzymes in fusariose wilts (Gothoskar *et al.*, 1953, 1955; Winstead and Walker, 1953, 1954; Waggoner and Dimond, 1955; Lakshminarayanan, 1955; Kamal and Wood, 1956). A detailed study of the conditions for the activity or synthesis of pectic enzymes by the pathogen would, therefore, seem imperative for understanding the physiology of parasitism. *In vitro* studies of this nature, though not strictly comparable to *in vivo* conditions, would, nevertheless, provide valuable information about the conditions offered by the resistant and the susceptible hosts.

It need hardly be emphasized that heavy metals play a vital role in the physiology of the pathogen in controlling the key enzyme systems *in vitro* and *in vivo*. The important part played by the two pectic enzymes, pectin methyl esterase (PME) and pectin polygalacturonase (PG) in the fusariose wilt of cotton (Lakshminarayanan, 1955) necessitates a study of the influence of the heavy metal ions on these enzyme systems. Fe''' is found to favour the production of PME at an optimal level of 1 p.p.m. and inhibit its activity at higher concentrations (Subramanian, 1956 *b*). It has also been reported earlier (Lakshminarayanan, *loc. cit.*) that Fe''' significantly increased the toxicity of the culture filtrate of *F. vasinfectum* to cut shoots of cotton at a concentration of 10^{-3} M. It is apparent from this that Fe''' not only accelerates the formation of pectic enzymes but also augments the toxicity of the products of host-parasite interaction. Other ions like Zn, Mn, Cu and Mo influence, in differing degrees, the production and activity of PME (which is the more important of the two enzymes), all the ions being inhibitory at higher concentrations. It appears, therefore, that the quanta of free heavy metals present *in vivo* in the host plants would largely determine the production of these enzymes in sufficient concentration for effective enzymatic degradation of the host substrate. Furthermore, the activity of these enzymes is influenced to a considerable extent by the presence of these ions in the enzyme-substrate complex. Zn and Mn inhibit enzymic activity, whereas Fe''' increases it (Subramanian, 1956 *b*). The exact manner in which the heavy metals bring about such an inhibition or activation is not clearly understood. It has been proposed by Hellerman and Stock (1938), Smith (1953) and Najjar (1948) that the enzyme activation brought about by trace elements takes place by the formation of metal chelates with the substrates resulting in the metallo-substrates suitable for enzymic action. Klotz (1951) proposed an alternative interpretation of the metal catalysis in enzyme reactions, emphasizing formation of metallo-enzymes. Similarly, the inhibitory effect may also be attributable to one of these reactions, *i.e.*, formation of metallo-substrate unsuitable for enzymic action or formation of metallo-protein rendering it inactive.

Recent work of Lakshminarayanan (*loc. cit.*) on the distribution of pectin in resistant and susceptible varieties of cotton revealed that the resistant varieties accumulated much less pectin in roots which form the primary focus of infection compared to susceptible ones, thereby providing unfavourable conditions for infection. High root pectin content was correlated with susceptibility and low root pectin content with resistance. The pectic enzymes which were also shown to be partly adaptive were secreted in sufficient quantities only in the presence of an optimal concentration of substrate. It

would be of particular interest to mention the work of Subramanian (1956 *b*) wherein amendment of the soil with various concentrations of zinc progressively reduced the root pectin content in the susceptible variety of cotton K 2, thereby affording protection against the disease. In addition to the change it produced in the substrate concentration at the focus of infection, namely the roots, zinc also had a marked inhibitory effect on the pectic enzymes of the pathogen as mentioned earlier. This is further borne out by the estimation of PME activity in roots, shoots and leaves of healthy and inoculated susceptible cotton plants grown at various concentrations of zinc. It has been observed that in the healthy cotton plants grown in unamended soil PME activity was almost none, especially in roots and shoots, but in the inoculated series there was a significant increase in the activity showing that this enzyme is operative during infection. As the concentration of zinc increases, there is a slight rise in the PME activity followed by a steep decline at 50 p.p.m. and thereafter. In other words, zinc seems to play a dual role in that it not only reduces the substrate concentration at the focus of infection but also inhibits its degradation by the enzyme. A study of the respiratory status of susceptible cotton plants in Zn-amended soil revealed the depressing effect of zinc on tissue respiration. In the inoculated series, however, the rise in the rate of oxygen uptake was counteracted and the rate closely followed the normal healthy controls. This was further confirmed by *in vitro* studies (Lakshmanan, 1956).

The net effect of infection is an increased rate of oxygen uptake (Lakshmanan, 1955) but the effect of pure fusaric acid, a vivotoxin in the fusariose wilt of cotton (Laskhminarayanan and Subramanian, 1955; Kalyanasundaram and Venkata Ram, 1956), is one of inhibition. This indicates that fusaric acid is not the only factor responsible for the production of the disease syndrome. Many of the commercial chelating agents like 8-hydroxyquinoline and EDTA (ethylene diamine tetra-acetic acid) exert a similar influence on tissue respiration. This may be attributed to their ability to chelate with metallic ions like iron and copper which lose their coenzyme function. Both the inhibitory action of the chelating agents and the acceleratory influence of the neat culture filtrate of *F. vasinfectum* were reversed by the addition of heavy metals.

Increased permeability of the host cells has been reported by many workers in response to an uptake of toxic metabolic products of fungi (Gäumann and Jaag, 1947; Gäumann *et al.*, 1947; Gäumann, 1951; Thatcher, 1942), but there is no suggestion of destroyed osmotic function. Davis and Dimond (1954) suggest that polyphenols like gallic acid may be responsible for the increased permeability. Recently we have observed

a significant derangement in the uptake of ions in infected cotton plants. Spectrochemical analysis using the standard Lundegårdh flame (spark-in-flame) method, of cotton plants infected by *F. vasinfectum* showed an increase in the magnesium, calcium, iron and manganese and a decrease in the potassium content over the healthy controls, suggesting a derangement in the selective absorption of ions (Sadasivan and Kalyanasundaram, 1956). An increased conductivity was also recorded in wilt-infected plants as compared to the healthy controls which may be attributed to the accumulation of electrolytes due to primary changes in osmoregulation of the host cells (Gnanam, 1956).

Kalyanasundaram and Subba Rao (1957) have recently obtained further evidence to show that with rise in temperature (over a range of 32·5° to 37·5° C.) of incubation of *F. vasinfectum* infected wilt susceptible cotton plants, grown in a thermostatically controlled, ± 0·1° C., miniature glass-house (Sadasivan *et al.*, 1956) there is an increased accumulation of fusaric acid, whereas there is a decreased disease syndrome as evidenced by a fall in wilt index. It seems, therefore, that the increased toxin content with rise in temperature (which is logical when one assumes higher transpiration and uptake of solutes) would presuppose an increased metallic accumulation and consequent *in vivo* chelation, a point that has to be proved by bioassay or spectrochemical evaluation of ash samples of infected plants grown under varying temperatures. This work of Kalyanasundaram and Subba Rao (*loc. cit.*) further showed the presence of cystine in plants grown at 37·5° C.; cystine, however, was absent at the lower temperature of 32·5° C. The spectrochemical estimations indicated a higher quantum of heavy metals (including Fe) alkali and alkaline earth metals other than K and as fusaric acid is known to be potentiated *in vitro* in biossay in the presence of free Fe''', it is presumed that the lower wilt index recorded by these workers at the higher temperatures may be due to (*a*) the presence of cystine which may be chelated with Fe making it unavailable for potentiation of the toxin, or (*b*) the adsorptive substances like proteins and carbohydrates being present in greater quantity in the host at higher temperatures.

In view of the fact that the heavy metals control several key metabolic functions and act as potentiators, activators or inhibitors of several enzyme systems (Foster, 1949; McElroy, 1953; McElroy and Nason, 1954; Saraswathi-Devi, 1956) of the host/parasite, it would be necessary to interpret carefully the important changes effected in the host, in the light of overall metabolic derangements during pathogenesis.

There seems little doubt that much work still lies ahead in the understanding of the intensely interesting host-parasite relationship of plants against a background of metallic chelation with vivotoxins and I naturally look forward to stimulating research in this fascinating field.

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STUDIES ON HORMONE TREATMENT OF SUGARCANE

I. Pot Experiments with 2, 4–D

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I. Introduction

The beneficial effect of 2, 4–D application on the sugar content of cane, as reported by Beauchamp,[1–3] was amply confirmed by Chacravarti, Srivastava and Khanna[4–6] who adopted the technique of large-scale experimentation involving milling tests at the factory so as to eliminate sampling errors which appeared to have led to erratic findings in a number of cases.[7–11] The average improvement in sugar content resulting from foliar spray of 2, 4–D (Sodium salt) obtained by Chacravarti, Srivastava and Khanna (*loc. cit.*) amounted to 0·55 unit. More recently these authors,[12] in course of preliminary experiments on soil application of 2, 4–D (amine salt), have observed improvements of the order of 3 units. Side by side with these large-scale trials, pot culture studies have also been in progress over a period of 2 years, the results whereof are presented in this paper.

II. Experimental

Large concrete drums ($2\frac{1}{4} \times 2\frac{1}{4} \times 4$) were used in the experiments. In both years, variety used was B.O. 11 and number of replications three. Treatments in the two years were as follows:—

First year

A: Control (no treatment).

B: 3 lb./acre 2, 4–D (Sodium salt) at planting.

C: 6 lb./acre 2, 4–D (Sodium salt) at planting.

D: 9 lb./acre 2, 4–D (Sodium salt) at planting.

E: 3 lb./acre 2, 4–D (amine salt) at planting.

F: 6 lb./acre 2, 4–D (amine salt) at planting.

G: 9 lb./acre 2, 4–D (amine salt) at planting.

Second year

A: Control (no treatment).

B: 6 lb./acre 2, 4–D (amine salt) applied 2 weeks after germination.

C: 6 lb./acre 2, 4–D (amine salt) applied at earthing up.

D: 6 lb./acre 2, 4–D (Sodium salt) applied 2 weeks after germination.

E: 6 sprays of 2, 4–D (Sodium salt) as 50 p.p.m. solution at 60 gallons per acre applied during July-September.

F: 1 spray of 2, 4–D (Sodium salt) as 50 p.p.m. solution at 60 gallons per acre applied 20 days before harvest.

G: 6 sprays of 2, 4–D (amine salt) as 50 p.p.m. solution at 60 gallons per acre applied during July–September.

In both years, basal manuring was done at 60 lb. N + 75 lb. P_2O_5 per acre in the form of ammonium sulphate and single super. Soil application of 2, 4–D was made in the form of a dilute solution or suspension containing 0·15 per cent. of the acid. The entire crop was harvested (age 9 months in first year and 12 months in second year) and records taken of yield and chemical attributes. In the first year's experiment, the latter consisted of brix, pol. and purity of juice but in the second year, glucose and fibre determinations were added. Sugar per cent. cane was calculated with the approximate formula of Noel Deerr[13]:—

$$\text{Sugar \% cane} = \text{Pol. \% juice} \times \left(1 - \frac{4}{3} \times \frac{\text{fibre \% cane}}{100}\right)$$

The results have been presented below:

III. PRESENTATION OF DATA

First year.—The results will be seen in Table I. Treatment G records a significant increase in brix over control and treatments G, E and F in pol. per cent. juice. Purity rises under individual treatments, although large, fall short of the level of significance. Yields are significantly depressed under treatments C, E and F. As the above are not quite systematic, combined analyses have also been done so as to assess the average effects of 2, 4–D in the two forms. These indicate that brix under amine salt application is significantly higher than in case of both control and sodium salt treatment and the same holds good for pol. per cent. and purity of juice. With regard to yield, the contrary effect is observed, the amine salt causing a significant depression as compared to control and sodium salt treatment. The latter does not differ significantly from control in any respect. The magnitude

of improvement in juice quality resulting from application of amine salt of 2, 4–D is considerable, average increase over control being 1·56 in brix, 3·26 in pol. per cent. juice and 12·92 units in purity. Assuming a fibre content of 15 per cent. for both treated and untreated cane, the improvement in sugar per cent. cane would work out to a value of the order of 2·6 units. On the other hand, the yield is reduced by 40 per cent.

TABLE I

Showing Effects of Soil Application of 2, 4–*D* (*Sodium and Amine Salts*) *at Planting*

Characters / Treatments	Yield (lb./drum)	Juice analysis		
		Brix	Pol.	Purity
A ..	10·4	16·0	9·18	57·3
B ..	10·0	15·1	9·19	59·1
C ..	6·4	16·6	9·79	58·9
D ..	9·6	16·1	10·24	63·6
E ..	6·4	17·3	11·83	68·2
F ..	4·6	17·1	11·74	68·8
G ..	7·6	18·3	13·77	73·6
Mean for sodium salt ..	8·66	15·91	9·74	60·52
Mean for amine salt ..	6·18	17·56	12·44	70·22
C.D. at 5% (treatment mean) ..	1·94	1·34	1·43	12·50
C.D. at 5% (means of sodium salt *vs.* amine salt)	1·12	0·77	0·81	7·22

Second year.—The results incorporated in Table II would indicate that considering the treatments individually, effects are not significant for any of the criteria, notwithstanding the fact that the actual improvements in juice quality attributes resulting from most of the treatments are quite large. An examination of the average effects of (i) the sodium and amine salts as also those of (ii) soil and foliar application, however, leads to a different

TABLE II

Showing Effects of Soil and Foliar Application of 2, 4–D (Sodium and Amine Salts) at Different Stages

Characters / Treatments	Yield (lb./drum)	Juice analysis				Fibre % cane	Sugar % cane
		Brix	Pol.	Purity	Glucose		
A ..	11·7	20·6	17·01	82·4	0·80	15·16	13·27
B ..	8·6	21·6	19·28	88·0	0·31	15·05	15·14
C ..	6·1	21·7	19·63	90·3	0·44	14·51	14·80
D ..	8·3	22·0	19·83	90·3	0·48	17·38	15·24
E ..	7·1	21·8	18·58	87·2	0·27	16·80	14·41
F ..	8·1	21·5	19·00	88·5	0·66	13·91	15·48
G ..	5·6	22·3	18·64	83·5	0·53	17·29	14·55
Mean for sodium salt ..	7·84	21·8	19·14	88·7	0·47	16·03	15·04
Mean for amine salt ...	6·77	21·8	19·18	87·3	0·43	15·62	14·83
Mean for soil application	7·69	21·8	19·58	89·5	0·41	15·65	15·06
Mean for foliar application	6·93	21·8	18·74	86·4	0·49	16·00	14·81
C.D. at 5% .. (sodium or amine salt means *vs.* control and soil or foliar application *vs.* control)	Non-significant	0·71	1·46	8·16	Non-significant	Non-significant	1·42

picture. Thus, considered from the former standpoint, the average increases over control produced by both sodium and amine salts of 2, 4–D are significant in respect of brix, sucrose per cent. juice, purity and sugar per cent. cane. Responses in cane yield, glucose per cent. juice and fibre content of cane are, however, non-significant. As between the average effects of sodium and amine salts, no significant differences are observed in respect of any attribute. The degree of improvement in quality brought about by both sodium and amine salts is considerable, being 6·3 and 4·8 units respectively in purity and 1·77 and 1·56 units in sugar content of cane.

On considering the results from the standpoint of average effects of 2, 4–D as soil and foliar application, irrespective of the form of salt, a similar behaviour is indicated. Thus both soil and foliar applications produce significant increases in brix, sucrose per cent. juice, purity and sugar per cent. cane as compared to control but they do not differ significantly between themselves. Yield, glucose and fibre values are not significantly affected. Improvements in quality attributes are quite large for both soil and foliar applications, being 7·1 units in purity and 1·79 units in sugar per cent. cane for soil application and 4·0 and 1·54 units respectively for foliar application.

IV. Discussion

In the first year's trials, involving soil application of sodium and amine salts of 2, 4–D at the time of planting (before germination), the former produced no significant effects on yield or quality attributes. The latter, on the other hand, occasioned a significant increase of the order of 2·6 units in sugar per cent. cane which was accompanied by a significant depression in yield amounting to 40% over control. During the second year, when treatments consisted of both salts of 2, 4–D applied in soil or as foliar spray, average effects of both forms of the hormone as also of both methods of application, were significant with regard to quality attributes and non-significant for yield. The actual mean improvements resulting from sodium and amine salts were of the order of 1·8 and 1·6 units respectively in sugar per cent. cane while the corresponding mean increases due to soil and foliar application were about 1·8 and 1·5 unit. The above results would appear to leave little doubt on the efficacy of 2, 4–D in increasing sugar in cane notwithstanding the fact that, considered treatment-wise, many of the effects did not attain the level of significance which was presumably due to the degrees of freedom being too low in such a case. So far as yield is concerned, the first year's results would point to the inadvisability of soil applications of the amine salt at the planting stage as also the lack of response to sodium salt applied in the soil at planting, both in the matter of yield and quality. With soil applications two weeks after germination or at the time of earthing up considerable improvement in quality results, with no adverse effect on yield of the crop and this holds good for both forms of 2, 4–D. Similarly, foliar application of either form increases sugar without affecting the yield. All these results fall in line with the findings of large-scale trials in collaboration with the industry.[4–6, 12] Particular mention may be made of the trials on soil application of amine salt[12] wherein two applications at interculture and earthing up produced an improvement in sugar content of the order of 3 units, with no adverse effect on yield or growth criteria at harvest,

viz., (i) number of millable canes per clump, (ii) number of green leaves per cane, (iii) number of internodes per cane, (iv) height of cane and (v) weight per cane. In this connection, it may not be out of place to refer to the findings of Overbeek[14] and Audus[15] to the effect that sugarcane growth is not adversely affected by fairly high concentrations of 2, 4–D even at the early stages of the plant. Similarly, it is of interest to note the observations of Rege (private communication), who conducted an extensive series of large-scale trials on pre-harvest spraying of 2, 4–D in South India and came to the conclusion that the treatment, on an average, produces increase in sugar content of the order of 0·2 to 0·4 unit. The above effect under South Indian conditions, where the crop has a longer period of growth and, at the time of pre-harvest spraying, is much nearer the stage of maturity than is the case in North India, might be regarded as particularly encouraging. In a recent review by H. M. L.[16] on sugarcane research in the Bombay-Deccan, Kulkarni and Nadgauda are reported to have observed favourable responses to 2, 4–D, indole acetic acid and o-Et-N-Ph-carbamate. It would thus appear that phyto-hormones offer immense potentialities for the sugar industry in all parts of the country.

V. SUMMARY

(1) Pot culture studies on the effect of 2, 4–D on sugarcane have been reported in the paper.

(2) Soil application of 2, 4–D (amine salt) at planting increases sugar content by about 2·6 units while depressing yield by 40%. Sodium salt of the hormone applied at this stage has no significant effect on yield or quality.

(3) Soil application of both salts, either two weeks after germination or at the time of earthing up, significantly improves sugar content (by 1·8 units) with no significant effect on yield.

(4) The same holds good for foliar application, mean improvement in sugar being 1·5 units.

(5) When applied to soil two weeks after germination, at earthing up or as foliar spray, the two forms of the hormone afford the same order of improvement in sugar (1·6–1·8 units).

(6) The above results conform to the findings of large-scale trials in collaboration with the sugar industry, reported earlier.

VI. ACKNOWLEDGMENT

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Central Sugarcane Committee, to whom grateful thanks are due. The assistance rendered by Sri R. C. Acharya, M.A., in statistically analysing the data presented here is also acknowledged.

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"SPIKE" (A NEW) DISEASE OF SUGARCANE

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I. Introduction

Various types of dwarfing of stalk in sugarcane, of causes known such as Sereh, Fiji (virus), Leaf-scald and Gumming disease (bacterial), downy mildew (*Sclerospora sacchari*) and of unknown causes, *e.g.*, Dwarf disease in Queensland (Australia),[1] Dwarf or Multiple Bud disease of Louisiana (U.S.A.),[5] Leafy Tuft disease of Bihar (India)[2, 4] or undiagnosed disease recently recorded from Bombay (India),[3] have been reported from time to time.

During the course of routine survey of diseases of sugarcane in Bihar, a peculiar type of stunting of stalks locally known as *Mudia* (from Hindi word, *mud* = head) was for the first time noted in Co 419 in the neighbourhood of Haveli Kharagpur (Distt. Monghyr) in South Bihar on 22/23–8–1954. Subsequent observations made during the last two years showed that the malady had established itself and could be spotted without much effort from mid-monsoon onwards. Since it differs from most of the cane diseases, described so far, a brief report on this disturbance of growth of stalk seems to be of interest at this stage.

II. Occurrence

Observations made in that locality in September and December 1954, showed that this type of disorder was not of rare occurrence. In September 1955, it was found in 20 out of 161 plots examined (Table I). This year it was noted as early as 26/29–4–1956 in 7 clumps in one plot out of 174 plots covering 83·2 acres in 23 villages. Towards the end of June, the malady was present in 13 out of 115 plots observed. In the third week of July, there was not much difficulty in tracing affected stalks. The same type of disturbance in the growth of stalks was also found in Warisaliganj and Ghosi (both in Gaya District) in South Bihar respectively on 23/25–6–1956 and 7–8–1956, in the same variety. Co 313 and another variety locally known as Gorakhpuria (unidentified cane) were also found affected at the latter place.

TABLE I

Occurrence of " *Stunting* " *in Co* 419 *at Haveli Kharagpur* (*Distt. Monghyr*)

Date	Plots examined	Area examined (in acres)	Plots affected	Area affected (in acre)
22/23- 8-1954	..	..	6	1·9
19- 9-1954	..	..	4	..
23/24-12-1954	..	..	15	..
3/5- 9-1955	161	74·4	20	5·1
7-11-1955	27	16·7	1	0·2
27/29- 4-1956	174	83·2	1	0·2
23/25- 6-1956 (At Warisaliganj)	87	34·4	10	3·6
26/29- 6-1956	115	41·5	13	3·9
18/19- 7-1956	..	..	20	5·5

It is interesting to note that the malady occurs concentrated in patches usually situated at the corners or edges of the fields. In these pockets the percentage of affected clumps may be as high as 75%. They are seldom found scattered all over the field.

This disturbance in the growth of a stalk may appear early in season in the mother shoot after it has formed a few normal leaves (Fig. 1), in all the tillers produced in a clump (Fig. 2) or even in one stalk in a clump after it has formed a few internodes of normal size (Fig. 3). It may affect stalks in mid-season (Fig. 4) or late in season (Fig. 5).

III. SYMPTOMS

Stalks suffering from this malady show varying degrees of reduction in the size of leaves and internodes associated with sprouting of buds *in situ* in the portion affected.

In mild cases, the malady is spread out over a few internodes. The leaves have shorter laminae and sheaths. So are the internodes without any appreciable reduction in girth (Fig. 4). Sheaths being unable to encircle them completely are pushed away (Fig. 6). There is practically no sprouting

of primary* buds (Fig. 8 *f, g*) or only a few of them germinate to produce lateral shoots without any sprouting of secondary* buds (Figs. 4 *a, b, c*; 5 *a, b, c*).

In medium type of affectation, the internodes lose in girth value also (Fig. 8 *h, i, j, k*) and sprouting is more pronounced.

In severe cases, almost all the buds germinate (Fig. 5 *a, b, c*) and the secondary buds also sprout *in situ* (Fig. 7), with the result that top presents a bushy appearance (Figs. 5 *c*; 8 *a, b*). The leaves and internodes are so abruptly and drastically reduced in size that the upper portion of an affected stalk in earlier stages, *i.e.*, before the sprouting of buds, resembles a spike (Fig. 1).

Apart from the reduction in size of leaf and internode, and the germination of buds, the stalk is often found bent in the affected zone (Figs. 4 *a*; 8 *d, f, h, i*) and the leaves are uniformly dark green in colour with a bluish tinge and somewhat purplish at margins.

There was no discoloration, hypertrophy or hypotrophy on leaf-sheath, lamina, or midrib. Nor was there any sign of injury on rind. On splitting open, the cane did not show any abnormality either in pith or in vascular bundles.

In view of spike-like appearance due to this disorder, it is proposed to call it " Spike " disease of sugarcane.

In mild and medium types of disturbance, stalks appeared to recover and the leaves and internodes gained in size till they were normal from this point of view (Figs. 6; 8 *c, d, f, g*). A stalk after having recovered might again show this disturbance (Fig. 4).

Attempts made to reproduce the disease in 1955 were not successful. Dormant buds from heavily affected stalks did not germinate. This year, one-budded setts from bottom, middle and top portions of mildly affected stalks planted on 23-7-1956 produced mother shoots with distinct signs of the malady in the case of bottom setts, and of retarded growth from the middle buds as compared to those formed by the top ones (Fig. 9). There are thus indications of the causal factor being present in the affected stalks though in varying degrees of intensity in the three regions.

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* Buds on a tiller (a stalk emerging from below ground level) are termed "primary". These on germination *in situ* produce lateral shoots hereafter called "secondary" shoots with buds on them designated as "secondary" buds which on sprouting form "tertiary" shoots and buds.

IV. Acknowledgements

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Explanation of Photographs

Fig. 1. Abrupt and drastic reduction in the size of laminae and sheaths of a mother shoot which had normal leaves to start with (2-anna coin for comparison of size. Photographed on 19th July 1956).

Fig. 2. Mother shoot and tillers showing heavy type of disorder (Transplanted to a pot on 1st July 1956 and photographed on 5th July 1956).

Fig. 3. A clump with one affected stalk (extreme right) in which uppermost two internodes and leaves (both sheaths and laminæ) were reduced. (Photographed on 19th July 1956).

Fig. 4 *a, b, c* respectively the lower middle and upper portions of a stalk which was mildly affected early in season and recovered later. Only a few primary buds sprouted and internodes were shorter in the middle of cane. Internodes in the lower portion of *a* and middle portion of *b*, are more or less equal in size. The stalk was again affected in upper portion, from arrow upwards (Photographed on 29th December 1954).

Fig. 5 *a, b, c* respectively the lower, middle and upper portions of a stalk severely affected late in season. Almost all the primary buds of the stalk and secondary buds in its upper portion sprouted *in situ*. Internodes were normal in size in lower (*a*) and middle (*b*) portion, while in the upper portion (*c*) they as also leaves were very much reduced. This combined with germination of secondary buds gave it a bushy appearance at top. (Photographed on 29th December 1954.)

Fig. 6. Mother shoot showing recovery, tillers also affected. Laminæ and sheaths in the middle portion were reduced in size, while those in the upper portion were getting progressively larger. In the lower portion they were normal in size (Photographed on 19th July 1956).

Fig. 7. Beginning of sprouting of secondary buds at the upper node; at the lower, secondary buds started germinating almost simultaneously with the primary bud with the result that the secondary and tertiary shoots were more or less equal in size (Photographed on 29th December 1954).

FIG. 8. Stalks with various degrees of disorder arranged according to their age, the oldest being on the left (Photographed on 8th September 1955).

a, *b*, severely disturbed resulting in the sprouting of all buds in the upper portion.

c, *d*, *e*, *f*,s howing recovery; internodes in the upper portion were of normal size; internodes in affected zone reduced in length but not appreciably in girth. A few buds germinated but there was no sprouting of secondary buds.

g, *h*, *i*, showing recovery; internodes in the affected zone showing distinct reduction in girth.

j, *k*, no distinct sign of recovery still.

FIG. 9 *a*, *b*, *c*, the mother shoots, formed respectively by top, middle and bottom buds planted on 23rd July 1956 (Photographed on 17th September 1956).

FIG. 1

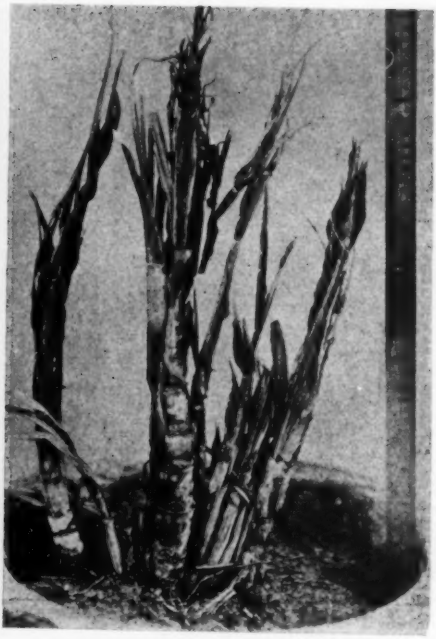
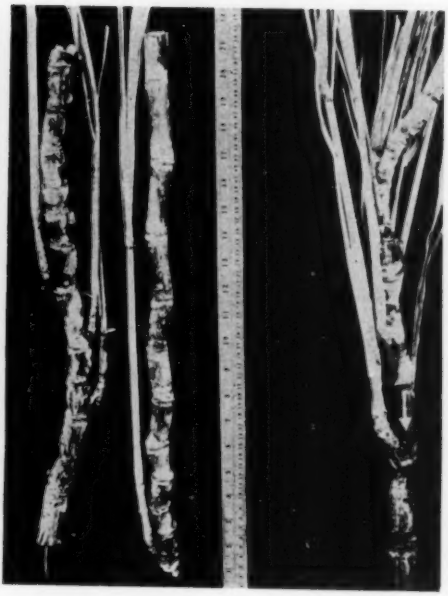


FIG. 2

FIG. 3



a b c

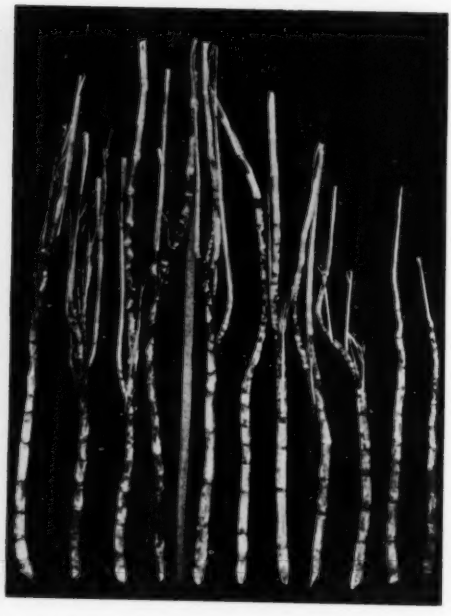
FIG. 4

a b c

FIG. 5

FIG. 6

FIG. 7



a b c d e f g h i j k

FIG. 8

a *b* *c*

FIG. 9

ON THE NATURE OF GROWTH INHIBITION OF *ESCHERICHIA COLI* BY AN OXIDATION PRODUCT OF VITAMIN B_{12}

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An interrelationship between *p*-amino benzoic acid (*p*ABA) and vitamin B_{12} has been suggested on the basis of observations that vitamin B_{12} (*i*) spares the *p*ABA requirement for growth of *p*ABA auxotrophs of *Escherichia coli* (Davis, 1951; Gots and Chu, 1952); (*ii*) potentiates *p*ABA action in reversing sulphonamide growth inhibition in *E. coli* (Shive, 1950); and (*iii*) is involved in the biosynthesis of certain metabolites in which *p*ABA plays a role (Alimchandani and Sreenivasan, 1955). The function of *p*ABA in the synthesis of these metabolites in *E. coli* is probably through a folic acid (FA) active compound since, it is observed that a combination of them could overcome growth inhibition of the organism by aminopterin (Shive, 1951).

A vitamin B_{12} oxidation product is known to act as an antagonist of this vitamin to micro-organisms under certain conditions (Beiler *et al.*, 1951; Rege and Sreenivasan, 1954). It was of interest to study the relationship discussed here between B_{12}, *p*ABA and FA *vis a vis* this antagonist in the metabolism of *E. coli*.

Experimental

E. coli (Macleod) was maintained by fortnightly transfer on peptone-yeast extract-agar slants.

The basal medium was that of Green and Sevag (1946). To 5 ml. lots of the double strength basal medium in bacteriological test-tubes the various additions as stated were made and the volume adjusted to 10 ml. with glass-distilled water. The tubes were capped and sterilised at 15 lb. steam pressure for 15 minutes.

The vitamin B_{12} oxidation product was prepared as described by Beiler *et al.* (1951). To 10 ml. of a solution containing 500 μg./ml. vitamin B_{12}, 5 ml. of concentrated hydrochloric acid and a few drops of 30 per cent. hydrogen peroxide were added. The resultant mixture was warmed, allowed to stand for 2 to 3 hours, neutralised with potassium hydroxide solution and

the volume adjusted to 50 ml. A control with water in place of the vitamin B_{12} solution treated similarly was kept alongside, and used always for comparison in all experiments with the B_{12} oxidation product.

A forty-fold dilution of a 24-hour growth of the organism in the basal medium at 30° was used to inoculate the tubes dropwise. Growth was measured turbidimetrically in a Klett Summerson photo colorimeter at 660 mμ and is expressed in terms of galvanometer deflections.

All results represent averages of three or four different sets of experiments.

RESULTS

In a preliminary experiment, the activity of the oxidation product was tested with *Lactobacillus leichmannii* and *E. coli* (Macleod). It was observed that the preparation inhibited growth of both organisms. The control preparation showed only a very slight inhibition with both the organisms indicating, contrary to reports (Hendlin and Wall, 1954; Lester Smith, 1954), that the activity of the product is independent of its sodium chloride or hydrogen peroxide concentrations. Vitamin B_{12} was effective in reversing growth inhibition of *L. leichmannii* but not of *E. coli*.

Since, as stated earlier, vitamin B_{12} may be functionally related to *p*ABA, the effect of the latter as well as of the metabolites in whose synthesis it is involved were tried with *E. coli* (Macleod) alone and in combination. These metabolites were (per 10 ml.): methionine (0·5 mg.), purine mixture (adenine, guanine and zanthine 0·1 mg. each), serine (0·2 mg.), thymine (0·25 mg.), valine (0·5 mg.), glycine (0·25 mg.) and vitamin B_{12} (10 mμg.) (Alimchandani and Sreenivasan, 1955). The concentration of B_{12} oxidation product used was equivalent to 30 μg. B_{12}/10 ml. which was just sufficient to inhibit growth completely. None of the foregoing metabolites, singly or in combination, could reverse this growth inhibition.

As B_{12} is involved in the synthesis of purine and pyrimidine desoxyribosides, the effects of deoxypentosenucleic acid and pentosenucleic acid and ammoniacal hydrolysates of these were tried (1 mg. each). The method of hydrolysis followed (Levene and Jacobs, 1910) is known to degrade the nucleic acid to the riboside stage. But no reversal could be observed.

When yeast extract (Difco), peptone (Bacto) or crushed cells extract of *E. coli* were used, it was observed that all of them possessed considerable activity in overcoming growth inhibition of the oxidation product. These supplements were also markedly stimulatory to the growth of the organism in absence of the antagonist (Table I).

TABLE I

Effect of yeast extract, peptone and E. coli *extract on growth inhibition of* E. coli (*Macleod*) *by* B_{12} *oxidation product*

Addition to 10 ml. basal medium	Growth at the end of 24 hours	
	With B_{12} oxidation product equivalent to 30 μg. B_{12}/10 ml.	Without B_{12} oxidation product
1. Nil	0	49
2. Yeast extract (Difco) (1 mg.)	106	132
3. Peptone (1 mg.)	95	120
4. *E. coli* crushed cells extract (1·2 mg. dry weight)	73	95

An attempt was made to ascertain the nature of this reversal of growth inhibition by the oxidation product.

Using mixtures of amino acids, purines and pyrimidines and vitamins of the B group, it was observed that the amino acid mixture alone was active in overcoming growth inhibition though only partially. The amino acid mixture also stimulated growth in the absence of the oxidation product (Table II).

TABLE II

Effect of amino acids, purines, pyrimidines and B vitamins on growth inhibition of E. coli (*Macleod*) *by* B_{12} *oxidation product*

Addition to 10 ml. basal medium	Growth at the end of 24 hours	
	With B_{12} oxidation product equivalent to 30 μg. B_{12}/10 ml.	Without B_{12} oxidation product
1. Nil	0	51
2. Amino acid mixture* (0·2 mg. each) ..	71	118
3. Purine and Pyrimidine† mixture (0·1 mg. each)	0	50
4. B Vitamins mixture‡	0	53

* Alanine, asparagine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, norleucine, methionine, norvaline, phenylalanine, proline, serine, threonine, tryptophane and tyrosine. In the case of the optically active amino acids the recemic forms were used.

† Adenine, guanine, xanthine, uracil and thymine.

‡ Thiamine HCl, riboflavin, nicotinic acid, pantothenic acid, pyridoxine HCl and *p*ABA (10 μg. each), pteroyl glutamic acid (PGA) (3 μg.), Leucovorin (LV), Lederle (6 μg.) and Vitamin B_{12} and Biotin (10 mμg. each).

With increasing concentrations of amino acid mixture increased reversal was obtained (Table III).

TABLE III

Effect of increasing concentrations of amino acid mixture on growth inhibition of E. coli (*Macleod*) *by* B_{12} *oxidation product*

Addition to 10 ml. basal medium	Growth at the end of 24 hours	
	With B_{12} oxidation product equivalent to 30 μg. B_{12}/10 ml.	Without B_{12} oxidation product
1. Nil	0	49
2. Amino acid mixture*—		
0·2 mg. each	69	117
0·4 mg. each	87	126
0·6 mg. each	99	127
1·0 mg. each	106	124

* Component amino acids same as in Table II.

Using the amino acids at 0·4 mg. each/10 ml., it was observed that, in spite of a ten-fold increase in the concentration of the oxidation product, no complete growth inhibition could be obtained (Table IV).

TABLE IV

Increasing concentrations of B_{12} *oxidation product and growth of* E. coli (*Macleod*) *in basal medium supplemented with amino acid mixture*

B_{12} oxidation product in terms of equivalent μg. B_{12}/10 ml. ..	Nil	30	60	100	200	300
Growth at end of 24 hours, in 10 ml. basal medium*+ amino acid mixture (0·4 mg. each)	128	89	77	64	54	47

* Component amino acids same as used in Table II.

When the amino acids were tried individually (0·4 mg. each) they were all found to be ineffective in overcoming growth inhibition by the B_{12} oxidation product at a concentration equivalent to 30 μg. vitamin B_{12}/10 ml.

Discussion

The considerably greater inhibition obtained with the oxidation product over that of the control preparation without vitamin B_{12} discounted the possibilities (Hendlin and Wall, 1954; Lester Smith, 1954) that inhibition was non-specifically caused by sodium chloride or by hydrogen peroxide.

The inability of B_{12} to overcome growth inhibition by the B_{12} oxidation product suggested that the B_{12} oxidation product was probably not just an antagonist of the vitamin. However, this failure to achieve reversal of the action of an antimetabolite in organisms which do not show a nutritional need for the metabolite has been known for glucoascorbic acid (related to ascorbic acid) (Woolley and Krampitz, 1943; Woolley, 1944), phenyl-pantothenone (analogue to pantothenic acid) (Woolley and Collyer, 1945) and hexachlorocyclohexane (related to inositol) (Fuller *et al.*, 1950). This may be explained as due to the fact that the analogue acts as an antagonist of a derivative of the vitamin, which is an intermediate more closely related to the functionally active form. It is also possible that the vitamin cannot be absorbed by the cell.

The inactivity of PGA, LV, nucleic acids, nucleic acid hydrolysates, *p*ABA and the metabolites in whose synthesis the latter is involved indicates that growth inhibition caused by the oxidation product is not due to an interference in the biosynthesis of these metabolites. The inactivity of PGA and LV, however, could be due to their not being available as FA source for *E. coli* (authors: unpublished data).

The activity of the amino acid mixture in partially reversing growth inhibition suggests that the oxidation product functions by inhibiting the synthesis of certain amino acids. The potency of the natural materials tried seems to be due to their contribution of these amino acids.

The inactivity of individual amino acids suggested that the biosynthesis of more than one amino acid is blocked by the antagonist. Presumably the oxidation product blocks the biogenesis of a common precursor of two or more amino acids.

Summary

1. An oxidation product of vitamin B_{12} inhibits growth of *E. coli* (Macleod).

2. Of various substances tried, it is observed that yeast extract, peptone, *E. coli* crushed cells extract or a mixture of amino acids, overcome growth inhibition of the B_{12} oxidation product to an appreciable extent.

3. The amino acids when tried individually are found to be inactive.

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REVERSAL BY FOLIC ACID OF PENICILLIN ACTION ON GROWTH AND ON PENTOSE NUCLEIC ACID SYNTHESIS IN *LACTOBACILLUS CASEI*

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Bellamy and Klimek (1948) reported that *Staphylococcus aureus*, when made resistant to high concentrations of penicillin, gave a negative Gram reaction. Gale and Rodwell (1948) confirmed this observation and showed that, parallel to development of resistance, the organism acquired an ability to synthesize its amino acid requirement. The Gram complex has been recognized to be a magnesium pentosenucleoprotein (Henry and Stacey, 1943; Bartholomew and Umbreit, 1945). Attainment of resistance to penicillin in this organism was in fact shown to be associated with a change in the general nucleic acid pattern in the direction of Gram negative organism (Gale, 1949). More directly, Mitchell (1949) has observed a disturbance in the nucleic acid-nucleotide balance as a result of penicillin action. George and Pandalai (1948) reported that penicillin inhibition could be reversed with nucleic acid although this was attributed to a lowering of pH on addition of nucleic acid to the growth medium (Ganapathi *et al.*, 1948). Cultural conditions favouring increased pentose nucleic acid (PNA) content of bacterial cells are known to enhance the resistance of *Hemophilus pertussis* cells to various antibiotics (Smolens and Vogt, 1953).

It was reported by Rege and Sreenivasan (1954 *a*), that the synthesis of both PNA and Deoxypentose nucleic acid (DNA) in micro-organisms is controlled by folic acid and vitamin B_{12}. These authors also showed (1954 *b*) that another antibiotic, aureomycin, inhibits synthesis of both PNA and DNA and that folic acid (PGA) exerts a protection against this effect; a higher concentration of vitamin B_{12} is required to exert similar adequate protection. The present report relates to a study of the interrelationship between these vitamins and penicillin with respect to their influence on growth and on nucleic acid synthesis.

Experimental

The organism used was *Lactobacillus casei* A.T.C.C. 7469 maint ined by fortnightly transfer on a medium consisting of (percentages): yeast

extract 1, glucose 1, sodium acetate 1 and agar agar 2·5. The basal medium was that of Tepley and Elvehjem (1945) devoid of peptone and purines.

In growth experiments, 5 ml. of double strength basal medium was taken in bacteriological test tubes, the various additions were made and the volume adjusted with glass-distilled water so that, on subsequent addition of penicillin solution after sterilisation, the volume would be 10 ml. The tubes were capped and sterilised at 15 lb. steam pressure for 12 minutes. After cooling, penicillin solution was added aseptically and the tubes were inoculated dropwise using a fifty-fold dilution of a 24-hour culture of *L. casei* in the basal medium containing 0·2 μg. PGA/ml. Growth was measured after 24 hours' incubation at 37° using a Klett Summerson photoelectric colorimeter at 660 mμ and is expressed in terms of galvanometer deflections.

The penicillin used was the crystalline sodium salt of penicillin G (Glaxo).

For studying the nucleic acid make-up, the organism was grown in Erlenmeyer flask containing 100 ml. double strength basal medium diluted after various additions to 200 ml. All additions except penicillin were made before sterilisation as in the growth experiments. The flasks were sterilised by autoclaving at 15 lb. steam pressure for 12 minutes. Each flask was seeded with 1 ml. of 18 to 24-hour inoculum.

After incubation at 37° for 24 hours, the cells were harvested by centrifugation, washed twice with ice-cold glass-distilled water, resuspended in water and made to a definite volume. Aliquots of this were taken for estimation of nucleic acids and for dry weight determinations.

PNA and DNA were extracted and estimated as described by Rege and Sreenivasan (1954 *a*) and expressed as averages of at least three replicates.

Since these studies relate to an organism requiring PGA for growth, the effects of B_{12} were ascertained only in presence of PGA.

RESULTS

Growth.—The concentration of penicillin to bring about approximately 50 per cent. grow h inhibition in presence of optimal concentration of PGA (0·3 mμg./ml.) was found to be 0·2 units/ml. Using this concentration of the antibiotic, the influence on growth inhibition of varying concentrations of PGA, with and without vitamin B_{12} was studied. Results given in Table I are averages of four different sets.

It is observed in Table I that PGA alone and, more effectively, with B_{12} affords protection against the bacteriostatic action of the antibiotic.

TABLE I

Effect of PGA and B_{12} on penicillin growth inhibition of L. casei

Additions to 10 ml. basal medium		Growth at end of 24 hours		
PGA mμg.	B_{12} mμg.	In absence of penicillin	In presence of penicillin	Per cent. growth inhibition
1	..	45	19	58·0
3	..	91	46	49·4
10	..	93	58	37·6
20	..	93	60	35·4
3	10	90	53	41·1
3	20	92	53	42·3
10	10	93	68	26·8
20	20	91	67	26·3

Nucleic acid make-up.—The nucleic acid contents of *L. casei* cells grown in presence of optimal concentration of PGA (0·3 mμg./ml.) with and without penicillin (0·2 units/ml.) are reported in Table II.

TABLE II

Effect of penicillin on nucleic acid content of L. casei

	PNA	DNA
	Per cent. dry weight of cells	
Without penicillin ..	9·54	2·08
With penicillin (0·2 units/ml.)	7·21	2·12

It is observed that *L. casei* cells grown in presence of penicillin contain decreased amounts of PNA, the amount of DNA being unaffected.

As in the growth experiments described earlier, PGA and vitamin B_{12} antagonise the bacteriostatic action of the antibiotic and as both these vitamins are known (Rege and Sreenivasan, 1954 *a*) to be involved in nucleic acid synthesis in micro-organisms, particularly *L. casei*, their effects on nucleic acid synthesis were studied (Table III).

TABLE III

Effect of PGA and vitamin B_{12} on nucleic acid make-up of L. casei *cells grown in presence of penicillin*

Supplements per ml. basal medium	DNA		PNA		
	With penicillin (0·2 units/ml.)	Without penicillin	With penicillin (0·2 units/ml.)	Without penicillin	Per cent. decrease
	Per cent. dry weight of cells				
PGA (0·1 mμg.) ..	1·64	1·47	4·36	7·21	39·0
PGA (0·3 mμg.) ..	2·25	2·20	7·30	9·62	24·1
PGA (2·0 mμg.) ..	2·61	2·75	8·24	9·80	15·9
PGA (0·3 mμg.) + B_{12} (2·0 mμg.) ..	2·90	2·81	8·76	9·73	9·9
PGA (2·0 mμg.) + B_{12} (2·0 mμg.) ..	3·35	3·48	9·29	10·08	7·8

The results indicate that folic acid alone and, more effectively, with B_{12} partially overcomes the antagonistic effect of penicillin on PNA in a manner similar to its protection against growth inhibition by the drug.

DISCUSSION

Penicillin is known to exert its effect during the logarithmic phase of bacterial growth (Hobby *et al.*, 1942; Boivin, 1947). This phase is usually associate with active synthesis of PNA (Boivin, 1947) and hence the growth-inhibitory action might be linked to an inhibition of PNA synthesis. The results outlined here demonstrate this influence of penicillin on PNA make-up of *L. casei* more directly. The work of Gale (1949) has also indicated such an effect in *S. aureus*.

PGA and vitamin B_{12}, which have been shown (Rege and Sreenivasan, 1954 *a* to control the synthesis of PNA and DNA in micro-organisms, are

found to counteract the inhibitory effect of penicillin on PNA. The role of the vitamins in the formation of the nucleic acids is presumably confined to the biosynthesis of the purine and pyrimidine moieties (Rege and Sreenivasan, 1954 *a*). The antibiotic may, in a similar manner, be acting by interfering in the formation of these bases. There are reports of penicillin having an inhibitory effect on pentose nucleases (Krampitz and Werkman, 1947; Massart *et al.*, 1947) and it is less likely therefore that the decreased level of PNA results from an accelerated breakdown consequent on the activation of the degradative mechanism(s) by the antibiotic.

Summary

1. Cells of *L. casei* grown in presence of penicillin are found to contain decreased amounts of PNA, DNA being unaffected.

2. PGA by itself and, more effectively, in presence of vitamin B_{12} exerts protection against the inhibitory action of the antibiotic on growth as well as on PNA formation.

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STUDIES ON THE ANATOMY OF SUGARCANE MOTH BORERS

I. Comparative Morphology of the Antennæ

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I. Introduction

Systematic studies of various moth borers of sugarcane have been taken up by quite a few workers in the past but while describing the external morphology of imagins, little attention has been paid to the antennæ.

The antennæ vary greatly in size and shape. Even the number of antennal segments vary from species to species. As such the characters are of great diagnostic and taxonomic value and therefore a detailed account of the antennæ is worthy of record.

Gupta (1940) observed that the antennæ of the males of *Chilotræa infuscatellus* (*Argyria sticticraspis*), *C. auricilia* (*Diatræa auricilia*), and *Chilo zonellus* are lamellate and flat and those of females are filiform. He further recorded the number of antennal segments of *C. infuscatellus* to be 41 both in male as well as in female moths. Kapur (1950) observed the antennæ of *Chilotræa* species to be with dark and whitish-brown rings whereas in the case of genus *Chilo*, that of the male are serrate and ciliate, and of the female setaceous. According to Imms (1951) antennæ is the most important character for differentiating family *Agaristidæ* from family *Noctuidæ*; the latter possessing one where shaft is not dilated. Jepson (1954) recorded that the antennæ of *Pyralidæ* vary in degree of pectination in the male, it being fine as compared to the one found in the *Agrotidæ*, where the antennæ in the male are strongly pectinate, bipectinate or serrate. He further opined that the antennæ offer characters which are of value in classification within genera.

II. Material and Methods

Eight species of moth borers of sugarcane, *viz.*, *Scirpophaga nivella*, (*Schoenobæini—Pyralidæ*)—*Chilo zonellus* (*Crambinæ—Pyralidæ*), *Chilo tumidicostalis*, *Chilotræa infuscatellus*, *Chilotræa auricilia*, *Bissetia*

steniellus (*Chilo trypetes*), *Sesamia inferens* (*Agrotidæ—Noctuidæ*), and *Emmalocera depressella* (*Anerastiine—Pyralidæ*) were selected for the present investigation. Over a dozen male and female specimens were examined in each species. Permanent slides of the head capsule along with antennæ were prepared by treating the specimens with 10% KOH solution, washing under a current of water, dehydrating, staining with Eosin in 90% alcohol, clearing in carbo-turpentine, turpentine and cedar-wood oil as suggested by Puri (1954) and finally mounting in turpentine-balsam.

To study the antennal segments, these were counted both on the binocular as well as on the research microscope to avoid chances of error. The number of specimens examined, of each species, varied from 24 to 32, the resultant observations being subjected to statistical analysis to determine whether or not the character could be used for purposes of identification.

III. Morphology of the Antennæ

Antennæ of the various species studied were in general long, slender and many jointed (Plate IV). There was very little difference in form and structure of the antennæ in the various species belonging to the same sub-family *Crambinæ—Pyralidæ* save in *Bissetia steniellus* where differences both in male and the female as also in the number of antennal segments were prominent. The antennæ of species belonging to different sub-families, however, had distinct features to distinguish these clearly from each other. Accordingly the description that follows is lso given according to the sub-families.

(*a*) *Schoenobiinæ* (Pyralidæ)

Scirpophaga nivella.—The antennae (Plate IV, Fig. A) are yellowish brown in colour, length of each antenna being 5–8·5 mm. with an average of 6·8 mm.

Scape.—It is longest of all the antennal segments, thick, elongate and slightly curved, broader at its distal end, tapering towards its proximal end (Plate V, Fig. 1). There are two articular knobs in the form of internal projections of the distal region at its dorso-lateral angles, which articulate with the pedicel. The proximal end of the scape has a deep concavity and is completely placed within the antennal socket. The rim of the socket close to the eyes develops a knob-like projection called the antennefer, which articulates with the proximal concavity of the scape as shown in Plate VII, Fig. 4. This articulation is of the socket and ball type and affords a free movement to the scape.

Pedicel.—(Plate V, Fig. 1). It is small, broader distally and about ⅓rd of the length of the scape. Its basal rim is projected proximally and vertically below to fit into the concavity of the scape. The pedicel proximally possesses a peg-like outgrowth at the right dorso-lateral side, which fits into the cavity formed by the internal projection of the scape. On the left dorso-lateral side can be seen a concavity developed by the outgrowth of the pedicel into which fits the peg-like projection of the scape. Close to the left side of the proximal rim is present an internal ridge or apodeme which braces the pedicel internally. The articulation between the distal end of the pedicel and the following annulus of the flagellum is dicondylic.

Flagellum.—(Plate VI, Figs. 1 and 2). It consists of 39–47 segments, the number being usually higher in males than in females. The first few segments are somewhat cup-like structures while the rest are dorso-ventrally flat segments. Each segment is provided with sensory-pits and the setæ. Leaving a few basal segments, all the segments have a projected sensory tooth which gives the entire segment a serrated outlook. The apex of the flagellum at its distal end bears a sense cone (Plate VI, Fig. 2). There is practically no structural difference between the antennæ of the males and females except that the segments in the females are slightly longer than those of males.

(*b*) *Crambinæ* (Pyralidæ)

The antennæ of *Chilo tumidicostalis* (Plate IV, Fig. B), *Chilo zonellus* (Fig. D), *Chilotræa infuscatellus* (Fig. C), and *Chilotræa auricilia* (Fig. F) are darkish brown, yellowish brown, light brown and light grey in colour respectively and are somewhat similar in form and structure while the antennæ of *Bissetia steniellus* (Plate VII, Figs. 1 and 3) exhibit to a certain extent the phenomenon of sexual dimorphism within the sub-family *Crambinæ*. The antenna of this species is greyish brown in colour and measures from 7 to 9 mm., average being 7·5 mm. Each antenna is on an average 6 to 8 mm. long save that in the case of *C. tumidicostalis*, which is slightly longer and measures from 7 to 9 mm.

Scape.—It is the shortest in case of *Bissetia steniellus* (Plate VII, Figs. 2 and 7) and longest in case of *Chilo tumidicostalis*. In *Chilotræa* species it is comparatively shorter, straight and narrow than in *Chilo* species, in which it is little more curved, long and wide. The peg-like projections causing articulations between the pedicel and the scape are present in all the species as usual.

Pedicel.—It is somewhat bulbous, concave distally, and projected proximally to fit into the concavity of the scape. The concavity of the pedicel

distally receives the convexity of the following annulus of the flagellum. The internal apodeme braces the pecidel internally. The antennefer in all these species occupies a position similar to that described in the case of *S. nivella*. In the case of *Bissetia steniellus* the internal apodeme is well marked and quite distinct as shown in Plate VII, Figs. 2 and 7.

Flagellum.—There is a well-pronounced difference between the flagel um of *Bissetia steniellus* and the rest of the species of this sub-family which have practically no difference amongst themselves. There is, however, a well-marked differentiation between the opposite sexes, *i.e.*, between the males and the females of all these species. Although the difference in the opposite sexes of these species is quite noticeable, yet the phenomenon of sexual dimorphism is not so well pronounced as in the case of *Bissetia steniellus* and other species like *E. depressella* and *S. inferens*. In *Chilotræa infuscatellus* (male) (Plate VI, Fig. 5), it is lamellate and flat while in others the antennal joints are more or less broadly rounded at the dorsal margin and are rather distinctly dilated laterally. The first few segments near the base are more or less cup-shaped, rounded and close in all the species. All the segments except a few at the base are provided with a tooth-like sensecone at the apex of one side, because of which the entire joints become serrate. In the case of *Bissetia steniellus* the joints of the male antenna are very broad and widely located, a character which differentiates the species from the rest of the species. This can be seen in Fig. 6 of Plate VII. The antennal segments in case of female antenna, however, are somewhat midway between the flat and serrated ones as shown in Fig. 8 of Plate VII. Majority of the segments constituting the flagellum in the females of other species are cylindrical and approximately of equal length (Plate VI, Figs. 6, 8, 10 and 11). Some other segments in female antennæ may present similar structure of joints as in the case of males as shown in Fig. 9 of Plate VI and Fig. 5 of Plate VII. In case of *Chilo tumidicostalis* and *Chilotræa infuscatellus* respectively. It appears necessary to apply this test of identification of sex rather carefully to the different species. It has, however, been observed that where the segments of the female antennae are somewhat similar to those of males, the joints in the female are comparatively narrow, a useful character to be borne in mind while differentiating between the sexes of such species. Gupta (1940) mentions the female antennæ of *Chilotræa infuscatellus* and other species as filiform which might be due to the study of various antennæ from the lateral view only instead of dorso-ventral one as shown in Fig. 9 and Fig. 5, Plates VI and VII respectively. In the lateral view the overlapping of the lateral sensory tooth-like cones become closely adhered thus giving filiform view of the entire flagellum. Sensory pits arranged in rows on

the joints may be comparatively more at the basal segments than on others which is most probably due to the absence of sense cones in case of basal segments. Setæ are also present on all the joints and besides, there is a sub-apical and sub-marginal row of cilia arranged laterally in all the species as shown in Figs. 5, 7, 12 (Plate VI) and 6 and 8 (Plate VII). The first joint next to the pedicel may be incompletely divided to appear the longest segment in some cases as is generally found in female antenna of *E. depressella* (Plate V, Fig. 7). The entire flagellum consists of 39 to 54, 44 to 51, 50 to 58, 47 to 54 and 50 to 57 joints in *Chilotræa infuscatellus*, *Chilotræa auricilia*, *Chilo tumidicostalis*, *Chilo zonellus* and *Bissetia steniellus* respectively.

(*c*) *Anerastiinæ* (Pyralidæ)

Emmalocera depressella.—The antennæ (Plate IV, Fig. E) of *E. depressella* are the longest amongst the antennæ of all the sugarcane moth borers. It is light brown in colour and is 7 to 12 mm. long with an average of 9·8 mm. Phenomenon of sexual dimorphism is very well pronounced, there being great difference between the antennæ of male and female specimens. The antenna in the male represents a diagnostic feature for separating this species from other species of sugarcane moth borers.

Scape.—It is the longest than those present in other species of sugarcane moth borers as shown in Figs. 7 and 8, Plate V. It is a long bulbous, club-shaped, about 4–5 times the length of the pedicel and receives proximally the antennefer from the rim of the antennal socket articulating with its concavity whereas the distal end receives the projected portion of the pedicel into its concavity.

Pedicel.—The pedicel is short, rounded structure which is projected below to fit into the concave surface of the scape. The distal end of the pedicel in male (Fig. 8, Plate V) is concave, hollow and receives a long antennal shaft, which does not seem to have been noticed and described by any of the previous workers. *Flagellum*: It can be said to consist of two distinct parts. The basal one which is about twice the length of the scape, consists of nine or more imcompletely divided and fused joints. The small conical comb-like pectinations at one of its lateral sides prove and support the imcomplete division of joints. There is also a well-marked depression on the other lateral side which results into an outward projection or convex surface of the side with comb-like pectinations. In this depression are observed scales and hair arranged in bunches to form brush-like structures, arranged in rows (Fig. 8, Plate V). A careful examination of these structures revealed that these may be sensory in function because of the presence of conical teeth-like structures. Moreover its existence in case of males only

probably seems to be connected with some sexual affinity for females and this aspect calls for further work to ascertain their importance and function. The remaining part of flagellum has got two types of joints the pectinated as well as the serrated ones. All the joints have a cylindrical shape with dicondylic articulations. A long comb's tooth-like or vertebral spine-like process comes out just as a lateral outgrowth from the middle of each segment on one side only thus forming a unipectinate view of such segments. All these segments are provided with many setæ and sensory pits. Last about 11 to 15 segments (Fig. 13, Plate VI), have less degree of pectination and appear to be serrate. These segments are also provided with sensory pits and setæ. The distal segment at the apex as usual is conical and is provided with a few long setæ. The entire flagellum consists of 59 to 69 segments. In case of female antenna the scape is comparatively broader (Fig. 7, Plate V). It is about four times the length of the pedicel. Pedicel is a short bulbous structure similar to that of other female antennæ of the *Pyralidæ*. The segment next to pedicel may be long segment with incomplete division into 3 to 4 segments as shown. The articulations of all the parts or joints of the antennæ are similar to those described in other *Pyralidæ* and the segments are somewhat cylindrical in appearance. There are 2 to 3 small star-shaped circles surrounded by minute setæ on many segments (Figs. 13, 15, Plate VI) a character which separates the female antenna of this species from the other female antennæ of the *Pyralidæ*. The entire flagellum consists of 45 to 65 joints.

(*d*) *Agrotidæ* (Noctuidæ)

Sesamia inferens.—The antenna is from 7 to 9 mm. long with an average of 8 mm. and is yellowish brown in colour (Plate IV, Fig. G). Sexual dimorphism is well pronounced like the antennæ of the *E. depressella*. The antenna in the male itself is a diagnostic feature for the identity of this species from other species of the sugarcane moth borers.

Scape (Fig. 2, Plate V):—It is a flat, thick and elongate structure, comparatively broader at its distal end and tapering at its proximal end. The articulation between the scape and the pedicel is at the dorso-lateral edges.

Pedicel.—The pedicel as usual is a bulbous structure.

Flagellum.—This in the case of male antenna (Fig. 3, Plate VI) is of vertebral type, vertebration being complete right up to the last but one distal segment at the apex, when studied dorso-ventrally. Previous workers like Krishnamurti and Usman (1952) described the antenna of *Sesamia inferens* as bipectinate because the lateral outgrowths arise from the base of the cylindrical segments but the dorso-ventral view of the joints clearly look like

a portion of the vertebral column and the joints at the distal end look like the caudal portion of the vertebral column. Each individual joint when studied from its dorsal side is somewhat triangular in shape, the longest side of the triangle being placed horizontally at its distal end and provided with many sensory pits and the setæ. Laterally each joint has long setal bifurcations. In lateral view the last few segments may give a serrated view as described by Krishnamurti and Usman (1952). Each triangular joint is supported ventrically by a cylindrical segment which articulates with the segments on either side. The antenna may be called vertebral due to the following reasons:

(*i*) The lateral outgrowths do not appear to be comb-like as in the case of *E. depressella* and their ends are not conical. (*ii*) On the other hand, the entire flagellum is easily comparable to some portion of the vertebral column, the vertebration being right upto the last but one segment at the distal end. The articulation between the flagellar joints is dicondylic as usual. The conical segment at the apex is provided with a few long setæ. The entire flagellum consists of 52 to 62 segments.

Scape in the female antenna is almost similar to that of male and is about three times the length of the pedicel. Its proximal end receives the antennefer in its concavity and its distal end provides internal projections and concavity for the articulation with pedicel. The pedicel also is a cup-like structure. The basal segments of the flagellum are somewhat rounded and flat (Plate V, Fig. 2) while others (Fig. 4, Plate VI) are flat, squarish in shape provided with apical sense cones as in *Crambinæ*. All the segments are provided with sensory pits and setæ and rows of cilia laterally. The articulation between the joints of the flagellum is also dicondylic. Each antenna bears a conical segment at the apex of the distal end as usual. The flagellum consists of 50 to 62 segments. The female antenna of this species can be easily identified from the shape of its segments (Fig. 4, Plate VI).

III. Number of Antennal Segments in Different Species

An attempt was also made to separate these species on the basis of the number of antennal segments in each species. It was observed that while the number of antennal segments in family *Pyralidæ* varied from 39 to 69, those in the sub-families *Schœnobiinæ*, *Crambinæ* and *Anerastiinæ* it ranged between 39 to 48, 39 to 58 and 45 to 69 respectively. In *Agrotidæ* (*Noctuidæ*) the antennal segments varied between 50 to 62.

The average number of antennal segments in *S. nivella*, *C. tumidicostalis*, *C. zonellus*, *C. infuscatellus*, *C. auricilia*, *B. steniellus*, *S. inferens* and

E. depressella was 44, 55, 50, 45, 47, 54 and 61 respectively. The variation in number of antennal segments in different species is shown in Table I.

TABLE I

Particulars of species	Specimen examineds (No.)	Range	Mean	Standard error ±	Remarks
Scirpophaga nivella ..	24	39–48	44·0	0·56	
Chilo zonellus ..	26	47–54	50·19	0·45	
Chilo tumidicostalis ..	32	50–58	54·56	0·32	
Chilotræa infuscatellus ..	24	39–54	45·62	0·73	
Chilotræa auricilia ..	24	44–51	46·96	0·99	
Sesamia inferens ..	24	50–62	54·42	0·57	
Emmalocera depressella ..	24	45–69	60·96	0·95	
Bissetia steniellus ..	24	50–57	53·71	0·55	

The differences between mean of these species were statistically significant at 5% level in all cases except in *S. nivella* and *C. infuscatellus* whose antennæ can be easily distinguished from their structural descriptions described above, and in *Bissetia steniellus* to that of *S. inferens*, which can also be very easily separated out from the structure and form of their antennæ.

IV. Number of Antennal Segments in Relation to Sex

Having examined the number of antennal segments in various species, an attempt was further made to study relation between the two sexes within the species. It was observed that in the case of females the range of variation in number of antennal segments is wider than in the males, though in certain cases like *Chilotræa* and also in *Chilo zonellus*, a wider range was observed in case of males than in females. A detailed summary of the observations recorded is presented in Table II.

The differences between means of the sexes were statistically analysed for all the species and were found to be significant at 5% level showing thereby even in those species where there is no sexual dimorphism, sexes

TABLE II

Showing number of antennal segments in males and females of different species

Species	Sex	Specimens examined (No.)	Range	Mean	Standard error ±
Scirpophaga nivella ..	Male	12	44–48	46·0	9·41
	Female	12	39–46	42·0	0·63
Chilo zonellus	Male	14	48–54	51·5	0·51
	Female	12	47–52	48·67	0·47
Chilo tumidicostalis ..	Male	16	53–58	55·5	0·30
	Female	16	50–57	53·63	0·46
Chilotræa infuscatellus ..	Male	12	43–54	47·0	1·11
	Female	12	39–48	44·25	0·73
Chilotræa auricilia ..	Male	12	46–51	47·58	0·44
	Female	12	44–48	46·31	0·40
Sesamia inferens ..	Male	12	52–62	55·5	1·00
	Female	12	50–62	54·42	0·50
Emmalocera depressella ..	Male	12	59–69	63·0	0·83
	Female	12	45–65	58·75	1·51
Bissetia steniellus ..	Male	12	50–54	51·33	0·37
	Female	12	53–57	56·08	0·37

may differ in the number of antennal segments thereby making identification feasible.

V. SUMMARY AND CONCLUSIONS

1. The anatomy of the antennæ of eight species of sugarcane moth borers, *viz.*, *Scirpophaga nivella, Chilo zonellus, Chilo tumidicostalis, Chilotræa infuscatellus, Chilotræa auricilia, Bissetia steniellus, Sesamia inferens* and *Emmalocera depressella* has been studied and described in details.

2. Well pronounced phenomenon of sexual dimorphism has been observed to be present in cases of *E. depressella* (*Anerastiinæ—Pyralidæ*), *Sesamia inferens* (*Agrotidæ—Noctuidæ*) and also to some extent in *Bissetia steniellus* (*Crambinæ—Pyralidæ*).

3. It has been observed that in species where sexual dimorphism is well pronounced the antennæ are of great diagnostic and systematic value for the identification of those species.

4. The present studies have revealed that the number of antennal segments in a particular species is never constant but each species has its particular range within which the number of antennal segments may vary. It has also been observed that generally the number of antennal segments in males is higher than in the females.

5. The data presented in the above-mentioned two Tables I and II was statistically analysed and it revealed that the difference between both means of all these species except in cases of *S. nivella* with that of *C. infuscatellus* and that of their opposite sexes was significant at 5% level.

VI. Acknowledgments

This work forms part of the scheme of Sugarcane Research Fellowships donated by the Sugar Industry in Bihar and the author is indebted to the donors for the facilities thus created to carry out an investigation of this nature. His grateful thanks are also due to Shri K. L. Khanna, Director of the Central Sugarcane Research Institute, Bihar, Pusa, for suggesting the problem and guiding it besides going through the manuscript. Similarly he is also thankful to Shri D. K. Butani, Field Entomologist, for helpful suggestions in the writing of the manuscript. The help rendered by Shri B. P. Sharma, Shri R. P. Shrivastava and Shri R. C. Acharya in field work, photography and statistical analysis respectively is also acknowledged.

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Explanation of Plates

Plate IV

The Antennæ :—

A—*S. nivella.*
B—*C. tumidicostalis.*
E—*E. depressella.*
F—*C. auricilia.*

C—*C. infuscatellus* G—*S. inferens*.
D—*C. zonellus*.

PLATE V

The scape and the pedicel:—

1. *S. nivella*.
2. *Sesamia inferens*.
3. *C. zonellus*.
4. *C. tumidicostalis*.
5. *C. infuscatellus*.
6. *C. auricilia*.
7. *E. depressella* (Female).
8. *E. depressella* (Male).

PLATE VI

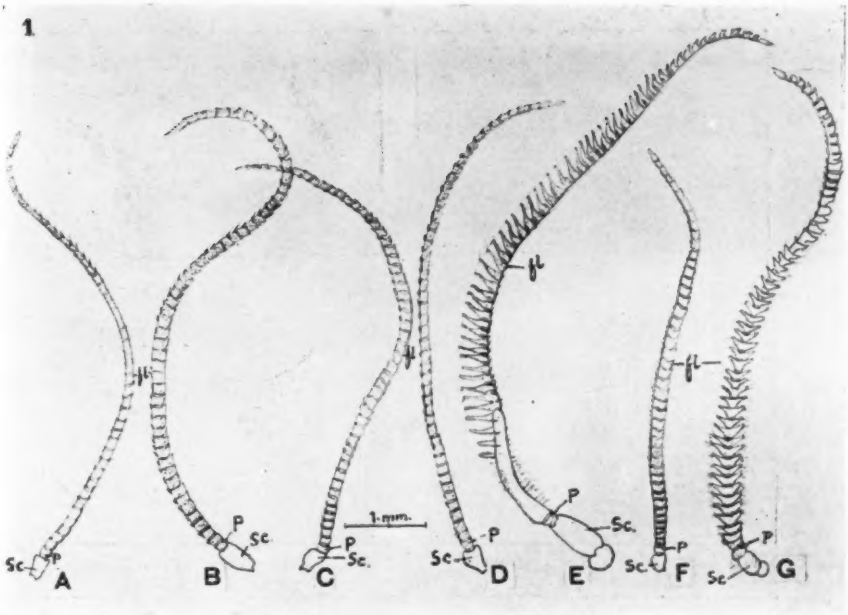
Portions of flagellum:—

1. *S. nivella*.
2. Tip of the same.
3. *Sesamia inferens* (Male).
4. *S. inferens* (Female).
5. *C. infuscatellus* (Male).
6. *C. infuscatellus* (Female).
7. *C. auricilia* (Male).
8. *C. auricilia* (Female).
9. *C. tumidicostalis* (Female). Dorsoventral view.
10. *C. tumidicostalis* (lateral view) (Female).
11. *C. zonellus* (Female).
12. *C. zonellus* (Male).
13. *E. depressella* (Distal portion—male).
14. *E. depressella* (Female).
15. *E. depressella* (Female).

PLATE VII

Antennæ and other parts:—

1. Male antenna: *B. steniellus*.
2. Scape and pedicel: *B. steniellus* (Male).
3. Female antenna: *B. steniellus*.
4. Portion of head capsule: *S. nivella*.
5. Portion of female antenna: *C. infuscatellus*.
6. Portion of male antenna: *B. steniellus*.
7. Scape and pedicel: *B. steniellus* (female).
8. Portion of female antenna: *B. steniellus*.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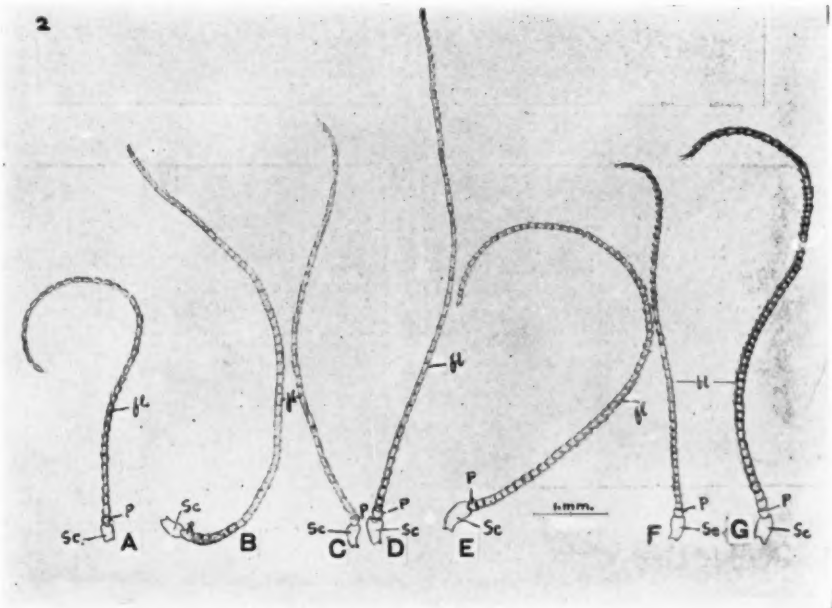
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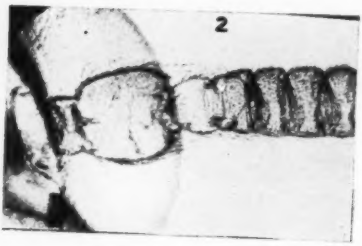
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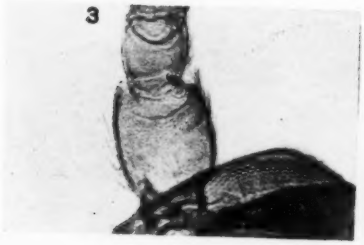
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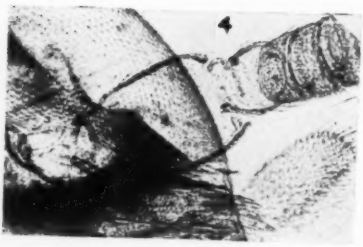
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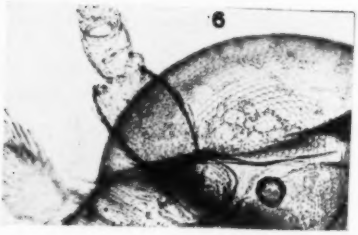
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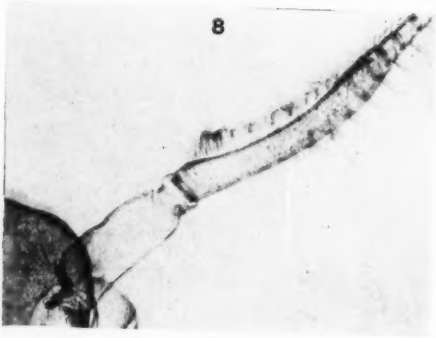
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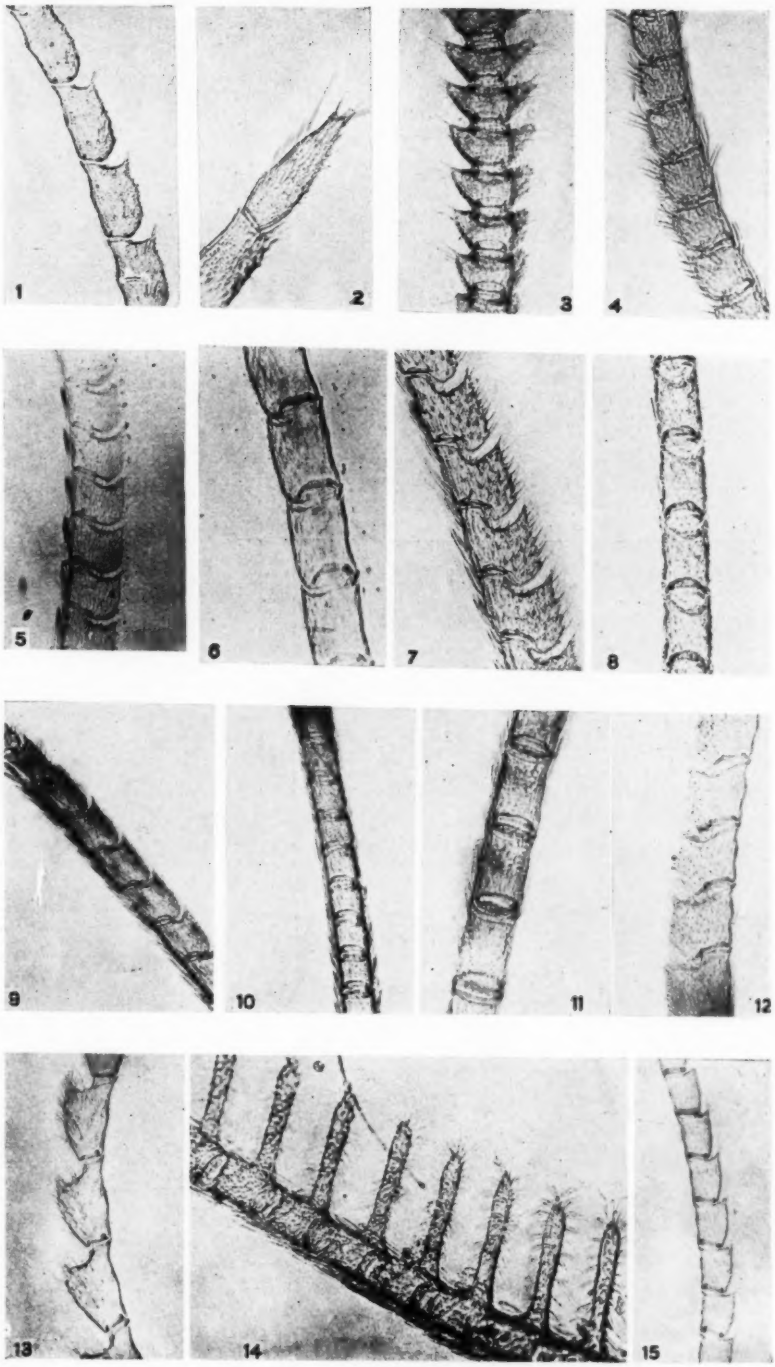
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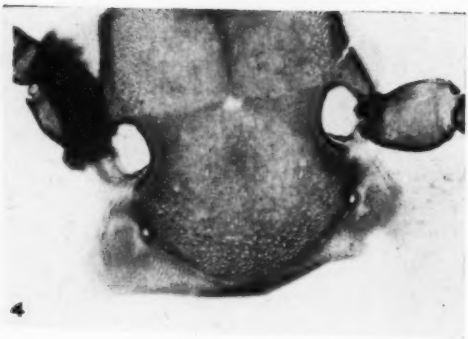
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CHEMICAL STUDIES ON INDIAN SEAWEEDS

II. Partition of Nitrogen

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Introduction

Several interesting studies have been made in the past on the nitrogen metabolism in seaweeds. Haas and Hill (1931) and Haas, Hill and Karstens (1935) isolated water-soluble peptides from the brown alga *Pelvetia canaliculata* and explained their presence as due to lack of metabolic balance traceable to either desiccation or low illumination. To obtain further evidence Haas, Hill and Russel-Wells (1938) examined the calcareous algæ *Corallina squamata* Ellis, *Lithophylum incrustans* Foslie, *Amphiora capensis* Aresch. and *Galaxaura subverticillata* Kjell. A number of unencrusted algæ were also examined, but so far among the latter group peptides were found only in the two species, *Pelvetia canaliculata* and *Griffithsia flocculoses*. It was found that the encrusted forms contained crude peptides to the extent of 0·05 to 0·29% of the dry weight.

Mazur and Clarke (1938) determined quantitatively the amino acids obtained by hydrolysis of formic acid extracts of a number of brown, red, green and blue-green algæ. Channing and Young (1952) attempted the partition of nitrogen in the Laminarialls, *L. saccharina*, *L. cloustoni* and *L. nodusum* and also in *P. canaliculata*, as volatile-N, nitrate-N, residual Kjeldahl-N and amino-N and reported values ranging from 0·07 to 0·24%, 0·03 to 0·92%, 0·58 to 1·8% and 0·58 to 1·3% respectively on the dry material. These authors also analysed the hydrolysates of the above algæ for the qualitative assessment of their amino acid composition. The major constituents found are aspartic acid, glutamic acid, glycine, alanine, valine, leucine, isoleucine, and other acids like serine, threonine, phenylalanine, lysine and arginine in traces. They could not find any difference between the amino acid composition of the two orders of the algæ studied. These authors (1953) again studied the amino acid composition of the acid hydrolysates of the brown algae, *L. saccharina*, *A. nodusum*, *P. canaliculata* and *Rhodymenia palmata*. Coulson (1953 *a*) has studied the nitrogen occurring

in free amino as well as in peptide forms in a number of seaweeds and reported differences between the various algæ examined. He (1953 *b*) also conducted qualitative studies on the amino acid composition of the proteins isolated from a number of marine algæ inclduding *Enteromorpha intestinalis*.

Takagi (1950) studied in detail the protein isolated from the Sea Lettuce (*Ulva pertusa* Kjell). The partition of the protein into water-soluble and alkali-soluble proteins was attempted and the arginine, histidine, lysine and cystine content of both fractions estimated. In another study (Takagi, 1951 *a*) the amino acids of *Ulva pertusa*, *Enteromorpha linza*, *Enteromorpha linza* var. *crispata*, all marine green algæ, were quantitatively studied by the two-dimensional chromatographic technique. The same author (1951 *b*) gives the nitrogen distribution in the various kinds of *Porphyra* during the different seasons. It is observed that the water-soluble nitrogen reaches a maximum in December with minimum in June. The author gives sufficient data to prove that there is considerable variation in the distribution of nitrogen in the various species of *Porphyra*, *viz*., *Porphyra tenera*, *P. umbilicalis*, *P. pseudolinearis*, *P. Okamurai*, *P. crispata* and *P. suborbiculata*. Takagi and Susuki (1952) estimated the sulphur-containing amino acids in a number of brown, red and green marine algæ and they found the largest amounts in the browns, next in the greens and least in the reds. No methionine was found in any of the species studied. Takagi (1952) separated adenine, histidine and lysine as monopicrates from *P. pseudolinearis*. Dewar (1952) refers to the work of Young on the amino acid constituents of *L. saccharina* and *A. nodusum*. The amino acids, glycine, leucines, aspartic acid, glutamic acid, serine, threonine, proline, phenylalanine and a trace of arginine were identified in the hydrolysates of the last two algæ. In a more recent investigation Ogino (1955) has studied the variations in the total, protein and non-protein fractions in a number of Chlorophyceæ, Rhodophyceæ and Phæophyceæ. He has shown that these fractions vary not only with the species and seasons but also with the age of the plants. Non-protein nitrogen fractions of these species have been shown to contain about 23 amino acids in the free state—the mono amino acids, glutamic acid, aspartic acid, alanine and cystine being the most common in most of the species while in certain species like *Prasiolo japonica* the diamino acid arginine was more conspicuous.

Besides these some amount of work has been done on the inorganic forms of nitrogen present in different types of algæ. Nitrates have been recognised in the growing apices of phæophyceæ by Suneson (1933).

Similarly in the Rhodophyceæ also nitrates have been detected by many workers (Kylin, 1915; Suneson, 1932).

In the light of the studies mentioned in the foregoing review on the nitrogen metabolism in seaweeds investigations were conducted on 11 species of seaweeds common to the Indian Coast. The species taken up for study, the place of their collection, the manner in which the samples were prepared for analyses, etc., have been dealt with in an earlier communication (Krishna Pillai, 1956). In the present account the data relating to the nitrogen constituents of the different seaweeds are presented. An attempt has been made to follow the seasonal variations in the oxidised inorganic nitrogen, volatile nitrogen, protein and non-protein nitrogen, and total and water-soluble organic nitrogen in the seaweeds. The amino acid composition of the acid hydrolysates of two sets of collections of the different algæ representing two distinct stages of growth has also been presented. The data bring to light the various changes taking place in each form of nitrogen during different growth stages of the plants as well as during different months. The knowledge is fundamental to understanding the physiology of the seaweeds.

Methods of analyses

Oxidised inorganic nitrogen.—Two grams each of the sun-dried seaweed were accurately weighed into a mortar and ground well with acid-washed sand. Nitrogen-free distilled water was added little by little during the process of grinding so that the entire material was converted into a colloidal mass. This was transferred to a beaker with the distilled water washings. The material was continually stirred with the aid of an electric stirrer for about an hour and finally centrifuged. The clear extract was taken in a conical flask and acidified with 10 c.c. of conc. hydrochloric acid. About 0·2 g. of Davarda's alloy was introduced, the flask loosely covered with a cork and left for a few hours and the contents were stirred occasionally. The solution was then filtered and the nitrogen estimated by the micro-kjeldahl method.

Total nitrogen.—Ten milligrams of the air-dried material were weighed into a kjeldahl flask and digested with 1 c.c. of conc. sulphuric acid in the usual manner using a mixture of copper sulphate and potassium sulphate as catalytic agent. After cooling the residue was taken up with distilled water and the ammonia estimated in the micro-kjeldahl. For the estimation of the protein-nitrogen quantities ranging from 10 to 20 mg. of the air-dried seaweed were used.

Water-soluble organic nitrogen.—One gram of the air-dried sample was ground well with acid-washed sand in a mortar and made into a fine paste with nitrogen-free distilled water. The pasty material was transferred to a beaker and stirred for an hour continuously. The mixture was centrifuged and the clear solution digested in the usual manner with sulphuric acid and estimated.

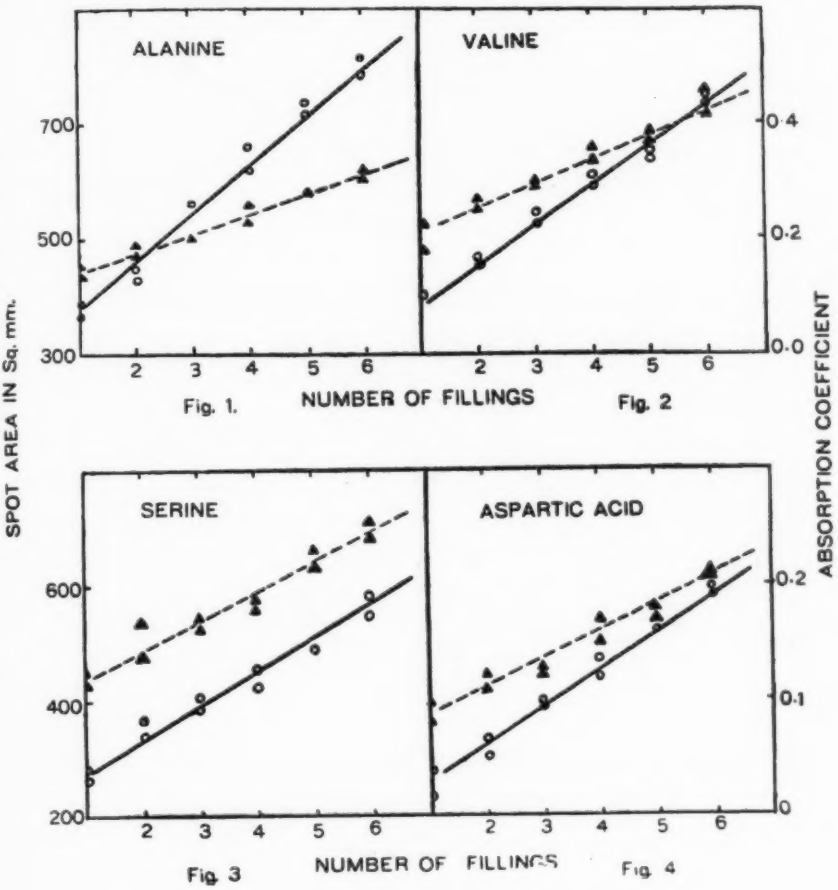
Volatile nitrogen.—Two grams of the air-dried sample were ground well with acid-washed sand. The pasty material was taken up with a few c.c. of water. The solution and washings were distilled in the micro-kjeldahl with alkali and the liberated gases were estimated by titration of the boric acid solution against N/70 sulphuric acid.

Amino acid composition of the seaweeds.—Two sets of seaweeds collected during August and December 1952 were examined for their amino acid composition. In the collection made in August the specimens were mature and fruiting while the samples obtained in December were young and non-fruiting. However, it could not be definitely said whether both collections belonged to the same parent stock, especially since some of the forms were of the floating type. But in spite of this defect the analyses give some idea of the differences in the amino acid composition of both the young and mature specimens of the different seaweeds.

Preparation of the hydrolysate for chromatography.—Two grams each of the air-dried sample were ground well with acid-washed sand and hydrolysed for 24 hours with 10 to 15 c.c. of 6 N hydrochloric acid. The hydrolysate was partially neutralised with sodium hydroxide solution and then concentrated in vacuum to a definite volume. The volume of all the hydrolysates is brought down to this level before microvolumes were spotted on chromatograms.

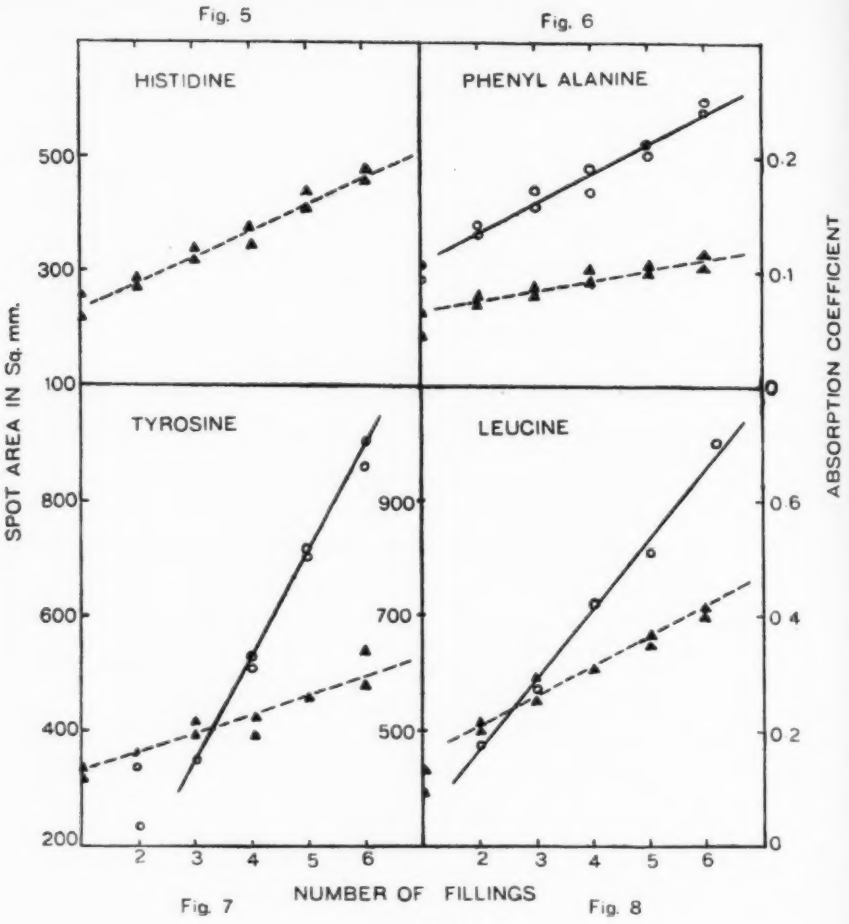
The chromatographic apparatus employed for this purpose was the one described by Krishna Pillai (1953). For the quantitative estimation both the area method (Viswanathan & Krishna Pillai, 1955) and the colorimetric method (Giri, Radhakrishnan & Vaidyanathan, 1953) were employed. Calibration curves for the spot area as well as the intensity of colour in presence of Cu^{++} ions on the spot content were separately drawn for each of the amino acids (Figs. 1 to 13). For determining the accuracy of estimation from unknown solutions standard mixtures of known amino acids were spotted and the corresponding amounts read off from the calibration curves for the spot area and the intensity of colour. It was found that the method (area method) is applicable to concentrations of amino acids between 20 and 100 micrograms and that the error is only within $\pm$ 10%. It is also

noticed that the area method is quite comparable to that of the method of Giri *et al.* where the colour of the spot is estimated.



FIGS. 1–4

For the estimation of the constituent amino acids of seaweed hydrolysates microquantities were spotted on Whatman No. 1 filter paper, developed in the solvent system, *n*-Butanol-acetic acid-water (4 : 1 : 5), and sprayed with ninhydrin solution. Standard mixtures of amino acids were also run side by side with the hydrolysates for purposes of easy identification. The spot areas were determined and the intensity of colour of the spots from a



FIGS. 5–8

duplicate set estimated in the absorptiometer. The results of analyses of the two sets of collections are given in Tables V and VI.

The monthly variations in the inorganic nitrogen content of the different species of seaweeds are given in Table I. Similarly the variation in the protein-nitrogen, water-soluble nitrogen and volatile nitrogen are given in Tables II, III and IV respectively. The actual values of total nitrogen, protein nitrogen, water-soluble nitrogen and volatile nitrogen of the different species are plotted in Figs. 14–22.

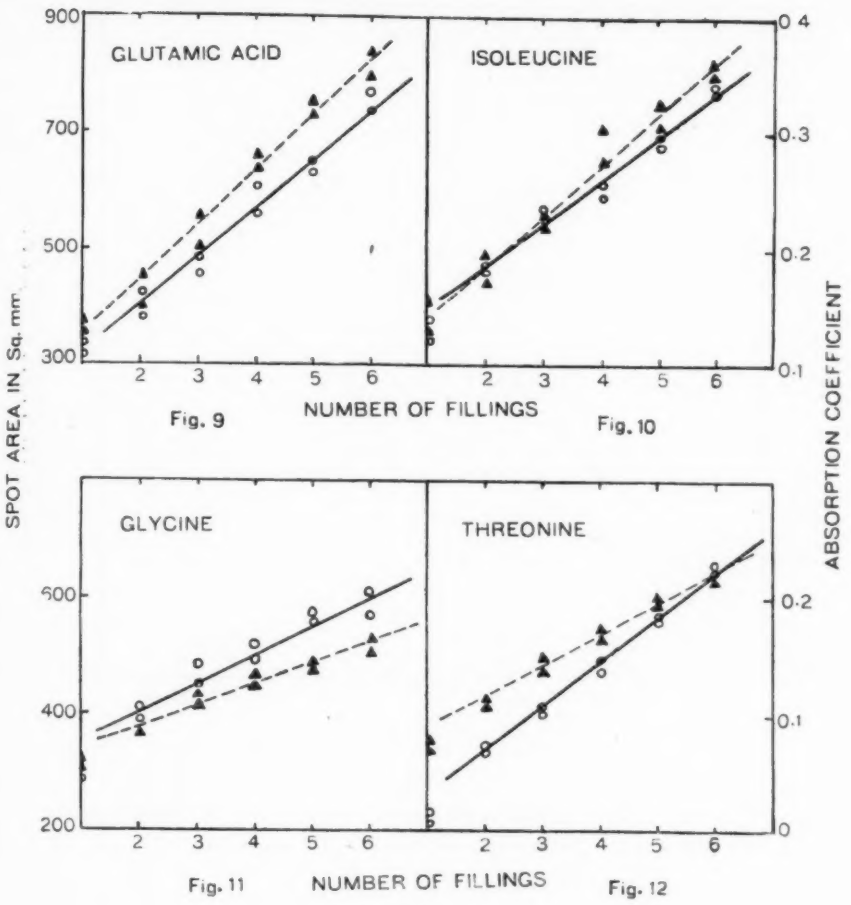


FIG. 9-12

DISCUSSION OF RESULTS

Oxidised inorganic nitrogen.—From the results it is evident that the maximum amount of inorganic nitrogen is found in the collections made in November, the period corresponding with the actively growing season of the algæ. This observation probably means that nitrate is present in greater quantities in the young fronds than in the older plants. The quantity of inorganic nitrogen decreases thenceforth at a slow rate until in some of the collections made in March–June the value falls to zero. A

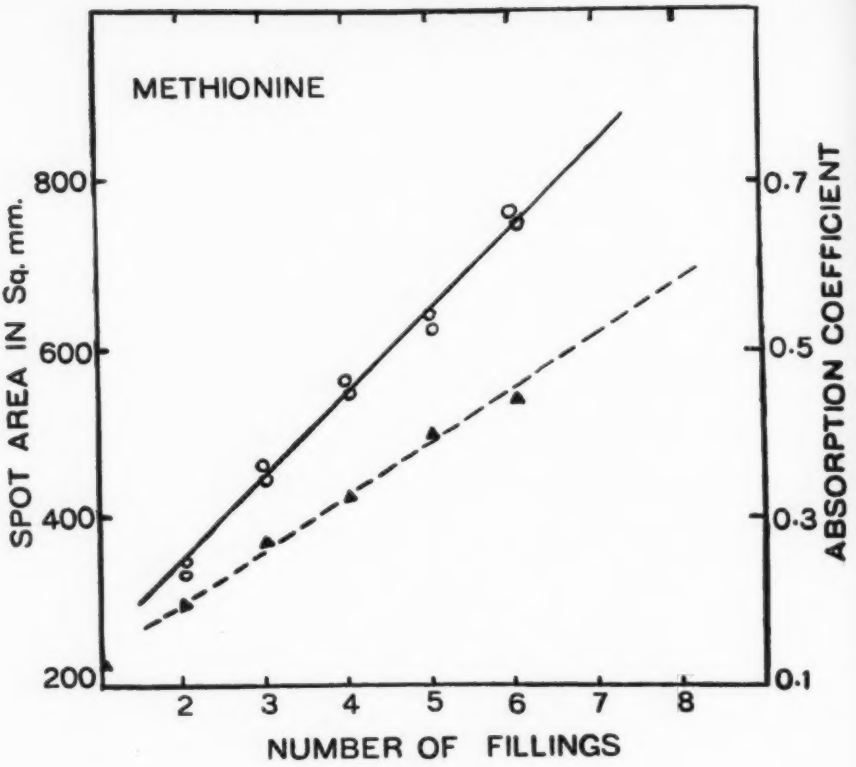


FIG. 13

FIGS. 1–13. ▲- - - - -▲ Absorption coefficient. O——O Spot area in sq. mm.
(1 filling of the micro-pipette contains 18·75 mg. of amino acid)

possible explanation for this phenomenon is that in the young plants nitrogen is absorbed from sea-water in the inorganic form and that as the plants grow the rate of nitrogen absorption from the water becomes less and less. The maximum value of inorganic nitrogen obtained is 0·047%N in *Sarconema furcellatum* in November, while other species examined give varying amounts (Table I). However, these values are small when compared with the figures reported by Channing and Young (1952) for *Laminaria* spp. and also with the values given by Suneson (1932) who reports 1·5% nitrate-nitrogen in *Ceramium rubrum*. With *A. spicifera* and *L. papillosa* another peculiarity is noticed. The inorganic nitrogen content in these two species never goes below 0·02%, whereas in most of the other species the minimum falls to

TABLE I

Variations in the inorganic nitrogen content in seaweeds

(Values expressed as percentage of dry matter)

Name of species	1952									1953		
	A	M	J	J	A	S	O	N	D	J	F	M
G. linum ..	..	..	..	..	..	0·018	0·021	0·030	0·020	0·020	0·019	0·013
G. lichenoides ..	Nil	Nil	0·009	0·010	0 010	0·011	0·019	0·021	0·014	0·015	Nil	Nil
C. dasyphylla ..	Nil	0·010	0·014	0·029	0·021	0·027	0·027	0·027	0·026	0·022	0·015	0·012
E. intestinalis ..	0·012	0·017	0·018	0·021	0·010	0·014	0·021	0·031	0·020	0·019	0·016	Nil
A. spicifera ..	0·023	0 022	0·020	0·021	0·022	0·031	0·036	0·036	0·031	0·030	0·021	0·020
L. papillosa ..	0·013	0·010	0·020	0·023	0·022	0·020	0·040	0·037	0·031	0·025	0·020	0·020
S. furcellatum ..	Nil	0·008	..	Nil	0·009	Nil	0·021	0·047	0·028	0·020	0·013	0·009
H. musciformis ..	0·014	0·000	0·009	Nil	0·015	0·012	0·017	0·018	0·014	0·016	0·009	Nil
R. intricata ..	..	..	..	..	..	..	0·041	..	..	..	Nil	..
P. australis ..	..	..	..	Nil	Nil	Nil	0·024	0·030	0·025	0·021	0·012	0·010

TABLE II

Variations in the protein-nitrogen in seaweeds

(Values expressed as percentage of total nitrogen)

Name of species	1952									1953		
	A	M		J	A	S	O	N	D	J	F	M
G. linum ..	33·7	74·6	64·5	78·7	63·4	55·6	37·5	50·7	36·1	42·9	..	..
G. lichenoides ..	69·5	64·2	67·6	77·1	98·1	68·2	78·9	60·2	25·4	32·4	58·3	41·9
C. dasyphylla ..	71·0	64·5	65·4	58·8	51·1	90·9	76·3	29·9	18·9	36·9	43·5	61·9
E. intestinalis ..	22·5	22·6	48·9	21·3	63·7	77·2	36·1	.	18·9	86·9	78·9	37·3
A. spicifera ..	63·2	48·1	48·3	59·5	72·9	92·4	76·9	..	20·6	38·6	..	66·1
L. papillosa ..	46·1	48·2	39·7	57·4	85·5	83·9	80·9	33·3	17·0	..	51·0	59·2
H. musciformis ..	35·6	42·1	63·4	63·8	64·5	89·4	83·3	59·3	20·4	24·1	43·6	60·0
S. filiforme ..	..	..	76·3	..	..	..	77·0	..	..	..	36·3	..
S. furcellatum ..	35·[illegible]	40·6	71·1	68·3	68·3	83·3	70·3	49·9	27·0	42·2	54·5	63·3
R. intricata ..	..	30 4	87·5	..	..	..	80·2	..	..	..	38·0	..
P. australis ..	..	27·0	45·1	43·4	59·9	85·4	79·8	67·9	75·5	68·7	60·1	44·6

TABLE III

Variations in the water-soluble nitrogen in seaweeds

(Values expressed as percentage of total nitrogen)

Name of species	1952									1953		
	A	M	J	J	A	S	O	N	D	J	F	M
G. lichenoides ..	16·3	17·6	21·6	37·3	68·9	40·0	62·8	60·0	42·7	41·1	37·1	58·1
C. dasyphylla ..	20·2	29·3	30·7	41·9	48·4	43·7	59·2	57·7	29·0	25·9	25·9	38·9
E. intestinalis ..	38·6	..	49·6	21·3	21·0	26·1	18·7	28·0	28·4	29·6	29·6	62·7
A. spicifera ..	67·7	61·6	55·4	53·3	52·8	36·0	32·3	..	28·8	29·0	..	33·9
L. papillosa ..	23·0	19·3	37·2	31·0	52·6	47·6	63·5	79·7	23·4	..	29·0	40·8
H. musciformis ..	40·7	24·0	35·2	38·0	55·6	43·6	65·5	69·1	29·2	24·0	19·4	40·0
S. furcellatum ..	14·9	27·1	..	18·4	58·9	54·6	72·7	80·2	33·7	37·6	29·2	36·7
P. australis ..	..	..	..	31·8	56·5	51·9	49·4	53·9	57·6	43·2	39·7	55·4

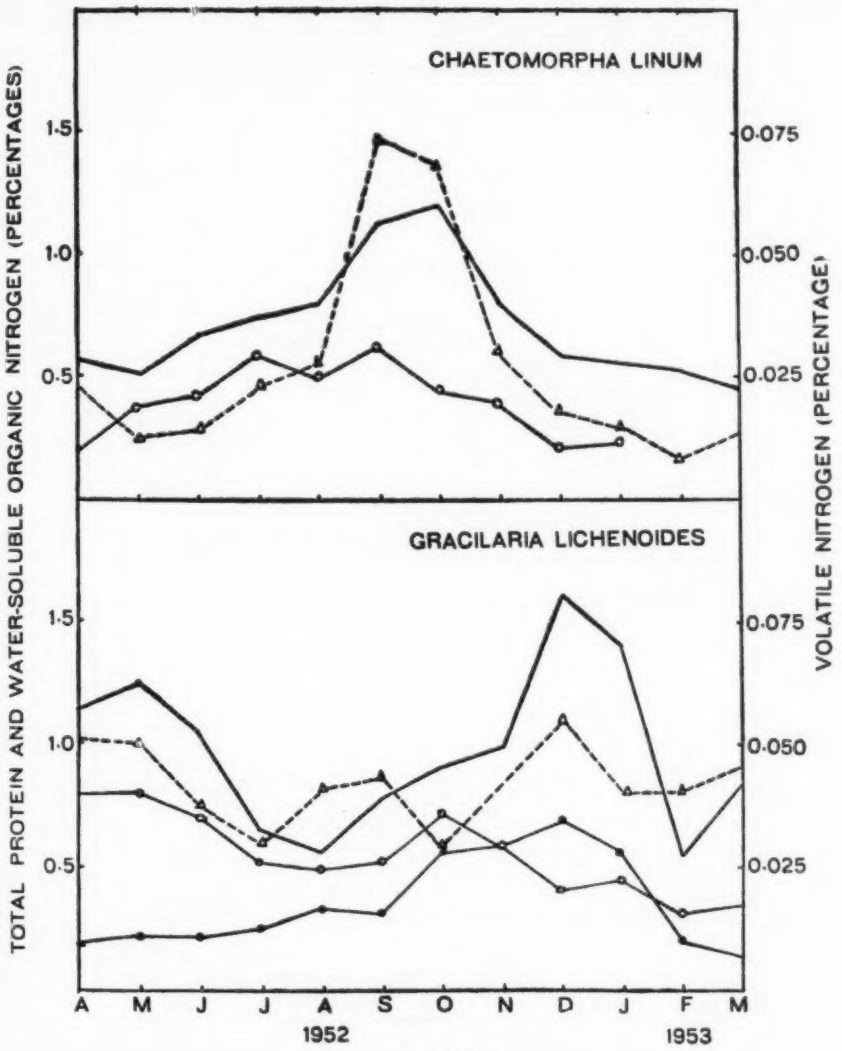
TABLE IV

Variations in the volatile nitrogen in seaweeds

(Values given as percentage of total nitrogen)

Name of species	1952									1953		
	A	M	J	J	A	S	O	N	D	J	F	M
C. linum ..	4·1	2·5	3·0	3·1	3·4	6·5	5·6	3·7	3·0	2·6	1·6	3·2
G. lichenoides ..	4·4	4·0	3·5	4·4	8·1	5·5	3·2	..	3·4	2·9	7·4	5·4
C. dasyphylla ..	4·1	3·4	3·7	2·8	2·5	5·0	4·0	2·4	2·5	2·5	2·7	2·6
E. intestinalis ..	4·6	6·8	5·7	3·8	5·2	5·3	3·3	3·1	3·1	5·6	5·2	1·8
A. spicifera ..	4·4	4·1	4·1	3·5	5·1	6·7	4·8	3·3	4·1	..	..	2·5
L. papillosa ..	5·3	4·9	5·5	3·1	5·2	5·0	2·7	2·7	3·8	..	2·5	3·6
H. musciformis ..	4·7	5·2	5·9	4·3	4·3	3·9	3·1	..	3·8	2·4	1·9	2·1
S. furcellatum ..	3·5	4·3	6·8	6·1	5·2	7·5	4·0	5·3	5·1	6·7	6·6	5·4
R. intricata ..	..	7·1	8·5	..	..	..	8·2	..	..	..	2·0	..
P. australis ..	5·7	5·6	5·7	5·4	4·3	5·0	4·8	5·6	7·9	..	3·0	3·3

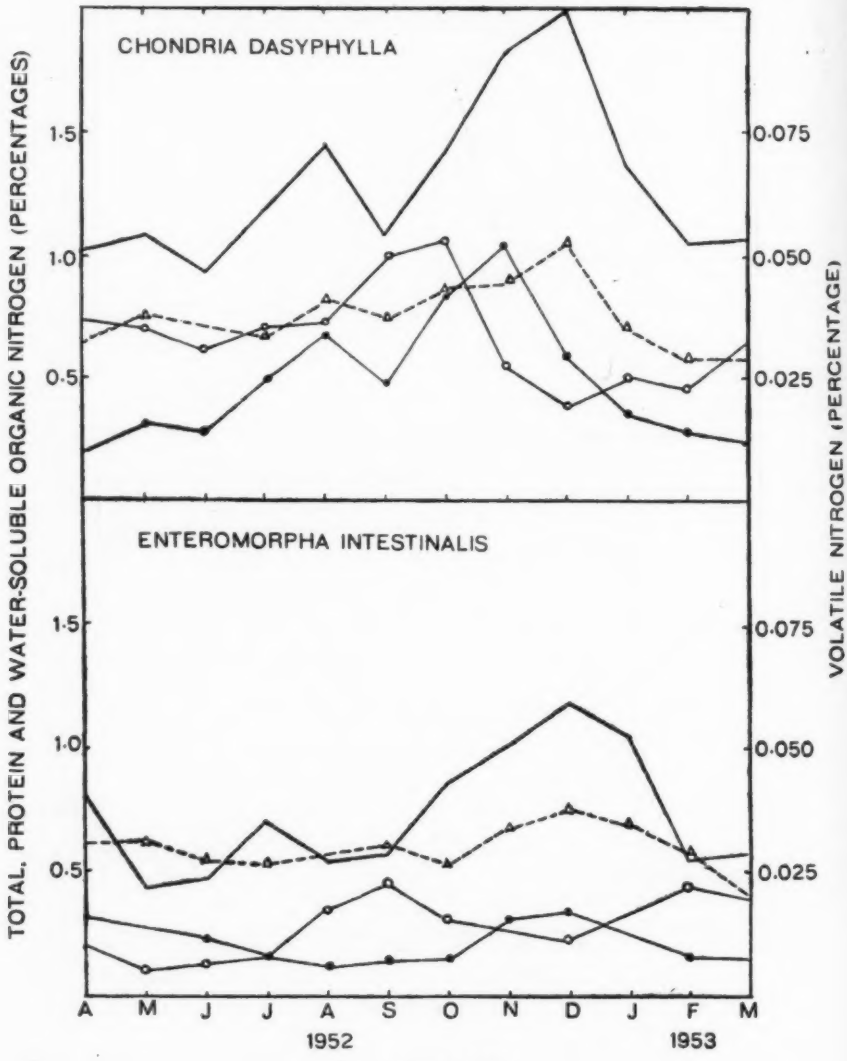
zero. This is an indication that in these two species the conversion of nitrogen to organic form proceeds only within certain limits.



FIGS. 14–15

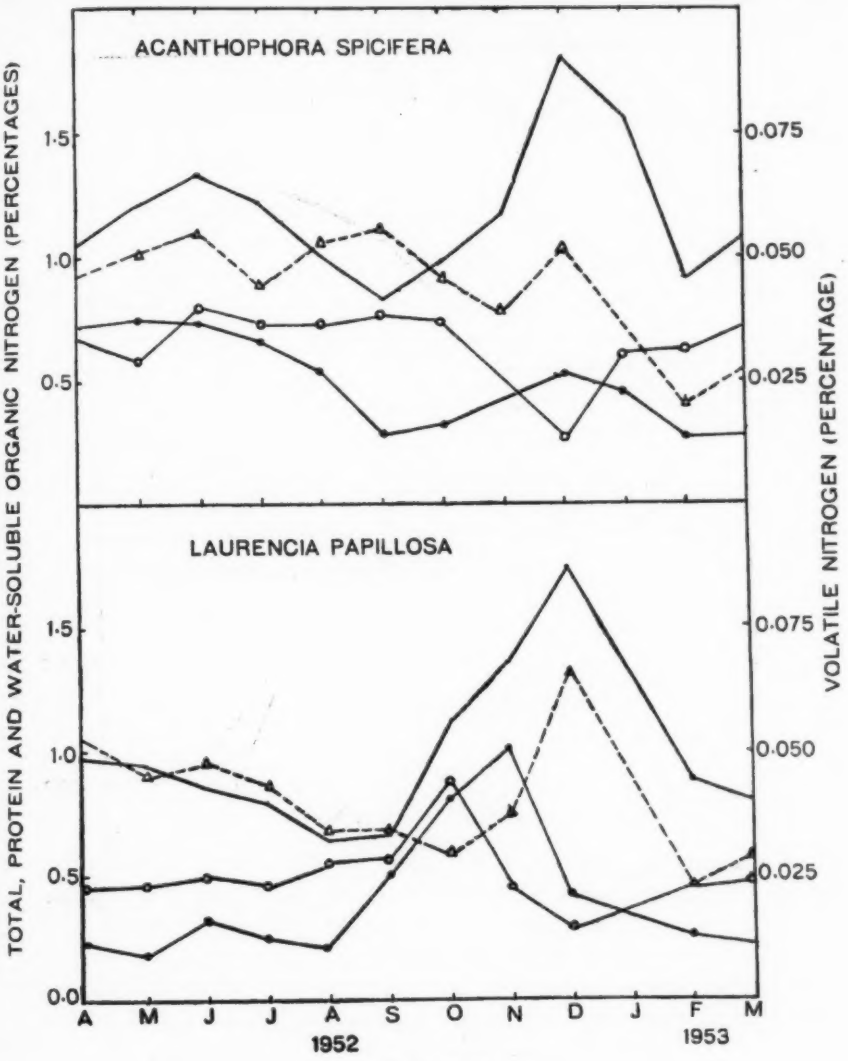
Total organic nitrogen.—The total organic nitrogen content shows wide variations reaching maximum values in October–December in most

cases. The minimum values are found in the months of June, July and August. The highest value 2·04% is recorded in *Chondria dasyphylla* (Fig. 16). In species like *G. lichenoides* the value for total organic nitrogen goes down to 0·5% in July–August. The usual variation in the total



FIGS. 16–17

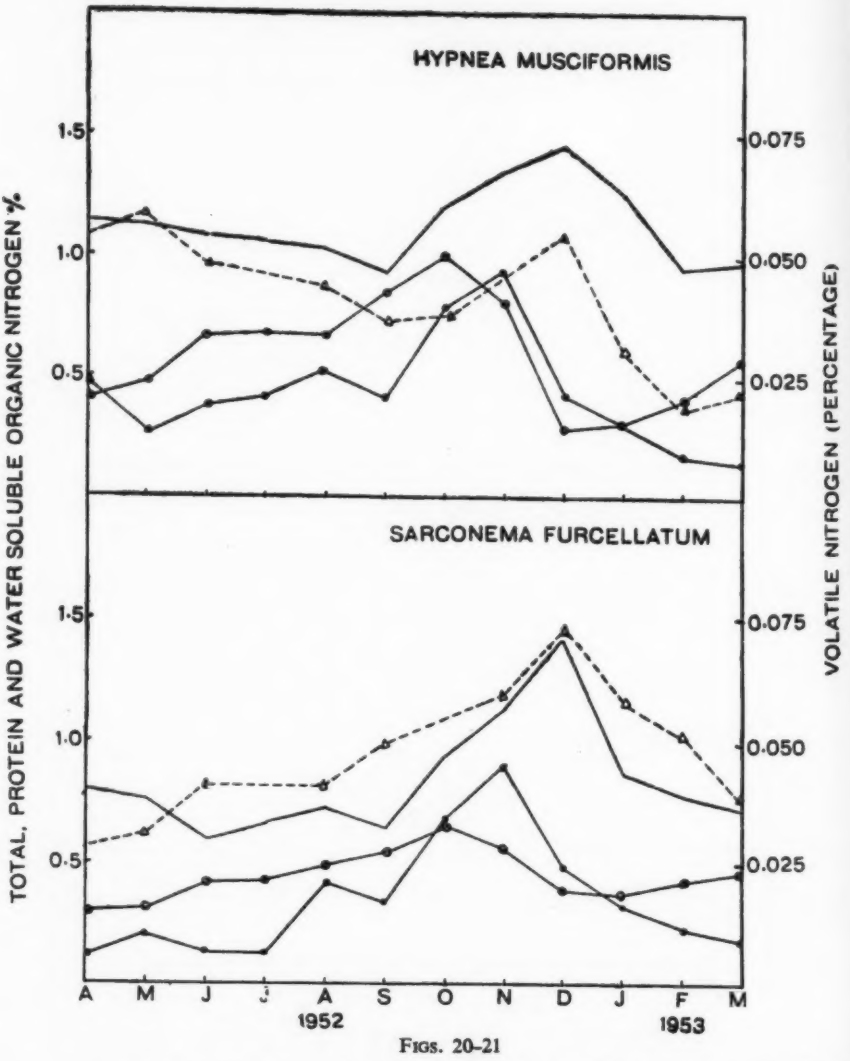
organic nitrogen content is between 0·5% and 1·5% of the dry matter. These figures are in perfect agreement with the values given by Channing and



FIGS. 18–19

Young though they have no comparison with the nitrogen values for *Porphyra* which contains about 8% total nitrogen. It may be noted that

the total organic nitrogen of the brown species is comparatively lower than that of the red, though maximum values of 1·02% and 1·29% respectively were obtained for *R. intricata* and *P. australis*.



FIGS. 20-21

Protein-nitrogen.—It can be seen from Table II that in the young plants collected in December when the total organic nitrogen is maximum the

protein-nitrogen is at a minimum constituting only 17 to 30% of the total nitrogen (an exception being *P. australis*). Probably at this stage the greater part of the organic nitrogen goes into the formation of chlorophyll in the rapidly growing algæ. Afterwards there is a slow increase in the protein nitrogen until it constitutes nearly about 70 to 90% of the total nitrogen in July, August and September. One striking characteristic is that the protein nitrogen is at a maximum when the total nitrogen is at a minimum (*vide* Figs. 14 to 22).

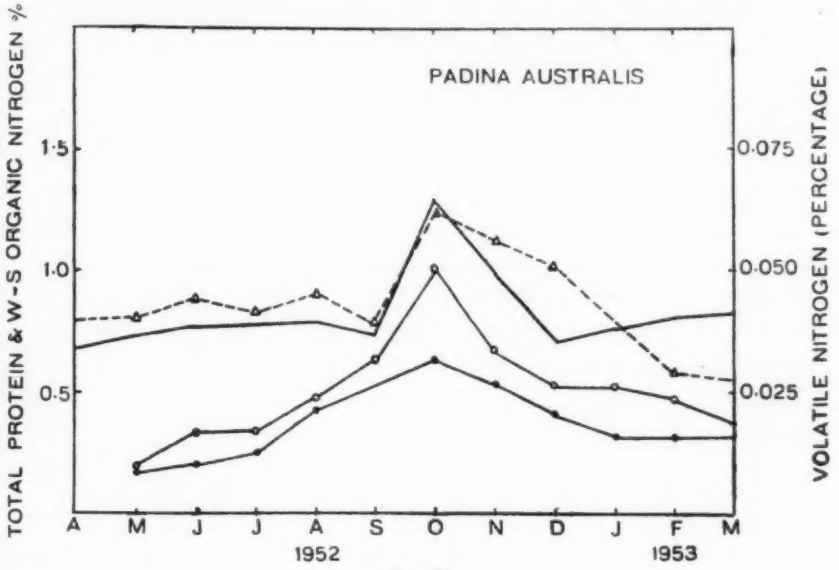


FIG. 22

FIGS. 14 to 22. ——— Total nitrogen. ○——○ Protein-nitrogen.
●— — ● Water-soluble nitrogen. △——△ Volatile nitrogen.

Volatile nitrogen.—From the figures plotting values of volatile nitrogen as percentage of dry matter content it can be seen that the amount changes with the total nitrogen giving maximum in October–December and minimum in June–July–August. But when the actual percentage of the volatile nitrogen on the total organic nitrogen as given in Table IV are considered it is seen that the maximum values are obtained when the protein is maximum.

Water-soluble nitrogen.—Water-soluble nitrogen also nearly follows the seasonal changes in the total organic nitrogen showing a maximum of about 60 to 80% of the total nitrogen in November in most cases (Table III). It

may be noted that the water-soluble nitrogen in the collections taken after December is equal or sometimes more than the protein-nitrogen especially in the case of the agarophytes. This probably means that the first stage of conversion of inorganic nitrogen is the water-soluble organic compounds. As the algæ grow the proportion of protein-nitrogen increases and the water-soluble nitrogen decreases thereby showing that in the mature plants the water-soluble nitrogen is converted to water-insoluble proteins.

Amino acids.—The seaweeds analysed for their amino acid content are: *G. lichenoides*, *C. dasyphylla*, *H. musciformis*, *A. spicifera*, *S. furcellatum* and *P. australis*. Table V gives the results of analyses done on the mature

TABLE V

Amino acid composition of the hydrolysates of mature specimens of seaweeds

(Values given as percentage of total nitrogen)

Amino acid	*G. lichenoides*	*C. dasyphylla*	*A. spicifera*	*S. furcellatum*	*H. musciformis*	*P. australis*
Phenylalanine	5·3	5·8	..	5·6	5·0	4·8
Leucine (*s*) ..	1·6	..	3·2	3·6	3·0	4·3
Tyrosine ..	2·5	4·0	..	3·2	..	2·8
Valine ..	4·2	3·2	4·6	4·0	4·0	4·0
Alanine ..	3·3	3·6	1·8	2·8	2·0	3·0
Glycine ..	1·8	2·0	2·0	2·0	1·8	2·0
Aspartic acid	1·5	1·3	1·6	1·5	1·5	1·6
Glutamic acid	3·2	3·4	2·0	3·0	2·8	2·4
Serine ..	1·8	1·2	2·0	1·8	1·6	1·2
Threonine ..	2·3	2·0	2·7	2·0	2·4	2·3
Arginine ..	7·4	7·0	6·6	7·6	7·0	7·4
Histidine ..	1·2	1·6	1·0	1·2	1·2	1·0
TOTAL	36·1	35·1	27·5	37·3	32·7	36·8

specimens and Table VI gives that of the young specimens. It may be seen that there is not much difference between the six different species of seaweeds in regard to the number of amino acids or in the proportion in which they

are present. Even though considerable difference has been noticed in the protein-nitrogen of the young and mature specimens of the various seaweeds the amino acid composition remains almost identical. This shows that the composition of the protein in both the young and mature plants is the same and does not undergo much change during growth.

TABLE VI

Amino acid composition of the hydrolysates of young specimens of seaweeds

(Values given as percentage of total nitrogen)

Amino acid	*G. lichenoides*	*C. dasyphylla*	*A. spicifera*	*S. furcellatum*	*H. musciformis*	*P. australis*
Phenylalanine	3·0	2·3	..	3·6	2·0	3–2
Leucine (*s*) ..	2·2	1·4	2·8	2·3	3·0	3·0
Tyrosine ..	1·0	..	..	1·0	1·0	1·8
Valine ..	2·3	1·8	2·0	2·3	1·6	2·6
Alanine ..	1·5	1·3	1·5	1·4	1·0	2·8
Glycine ..	1·0	1·7	1·0	1·0	1·0	2·7
Aspartic acid ..	1·0	0·8	1·2	0·8	1·0	1·8
Glutamic acid	2·4	2·0	..	2·2	2·2	4·3
Serine	1·0	0·8	1·0	1·0	0·8	2·3
Threonine ..	1·2	0·7	1·2	1·2	1·0	2·7
Arginine ..	4·3	3·7	4·0	4·8	3·8	6·3
Histidine ..	Nil	..	..	..	..	1·4

SUMMARY

An attempt has been made to study the chemical partition of nitrogen in eleven species of seaweeds belonging to the three major groups, Chlorophyceæ, Rhodophyceæ and Phæophyceæ. The seasonal variations in the total organic, water-soluble, volatile, protein and non-protein nitrogen contents were followed by analysing regular monthly collections of the algæ. The importance of each of the fraction in the metabolism of the algæ has been discussed. It is observed that the seaweeds are poor in their total nitrogen content, the values never exceeding 2% on the dry basis. In species

like *G. lichenoides* the total nitrogen content shows an inverse ratio to that of the agar content. Protein nitrogen is less in young plants, while in the mature plants it accounts for more than 75% of the total organic nitrogen. Water-soluble and volatile nitrogen content also follows the total nitrogen giving maximum values in September–December and minimum in July.

The amino acid composition of two sets of collections representing two distinct growth stages has also been studied quantitatively. It is observed that although the protein-nitrogen content varies substantially between the young and the mature plants the amino acid composition remains almost identical.

The chromatographic method employed in the quantitative estimation of the amino acids has been outlined.

ACKNOWLEDGEMENTS

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MECHANICAL AND ELECTRICAL RESPONSES OF UNSTRIATED MUSCLE IN SODIUM-FREE SOLUTIONS

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Overton (1902) demonstrated that frog muscles became inexcitable when they were immersed in isotonic solutions containing less than 10 per cent. of the normal sodium chloride concentration. He also showed that chloride ions were not essential constituent of Ringer's solution, since excitability was maintained in solutions of sodium nitrate, bromide, sulphate, phosphate, bicarbonate, benzoate, etc. Lithium was the only ion that could be substituted for sodium. Overton was unable to repeat his experiment with a frog's sciatic nerve, which maintained its excitability for long periods of time in salt-free solutions. This has been considered as due to retention of salt in the interstitial spaces of the nerve trunk. Thus Kato (1936) found that application of isotonic dextrose to single medullated fibres of the frog caused a rapid but reversible loss of excitability, and a similar result was obtained by Erlanger and Blair (1938) on the sensory roots of the bull frog. For other references see Hodgkin (1951).

Singh and Singh (1943) and Singh (1944) found that the stomach muscle of the frog, *Rana tigrina*, had the remarkable property, that it remained irritable in hypotonic (0·112 *M*) solution of sucrose for several hours. In the present research, these experiments have been extended to determine the role of sodium in excitability and action potentials; a preliminary report has been published elsewhere (Singh, 1957; Singh and Bhatt, 1957).

Experimental

In these experiments transverse pieces of the stomach muscle of the frog, *Rana tigrina*, were used. Some experiments were also performed on similar pieces of dog's stomach muscle, and on tænia coli of the guniea pig. The muscle was stimulated electrically with alternating current, 12 volts, 50 cycles, and direct current, 12 volts, by the method of Winton (1937). It was also stimulated with acetylcholine (1 in 100,000) and adrenaline (1 in million).

Sodium was estimated by the method of Weinbach (1935), as described in " Practical Physiological Chemistry " by Hawk, Oser and Summerson

(1949), and potassium by the method of Looney and Dyer (1942) similarly described. Preliminary digestion of the muscles was made with 50 per cent. nitric acid as described by McDowall, Munro and Zayat (1955).

In the sucrose solution, using the Cossor Oscillograph, Model 1049, the action potentials were recorded by thrusting two fine copper wires into the muscle at two points; they were recorded when the muscle showed spontaneous contractions, the mechanical response being also recorded at the same time.

The muscle was immeresed in about 100 c.c. of sucrose and other sodium-free solutions. This was changed every 15, or 30 or 60 minutes to remove sodium from the muscle, as thoroughly as possible. After immersion for 3, or 4 or 6 hours, when contracting strongly, the muscle was removed and analysed for sodium and potassium. The muscle usually weighed from 0·5 to 1 gm. and the maxiumm tension it develops when stimulated electrically is about 5 to 10 gm.

Results

Effect of sucrose.—In full tonic sucrose (0·224 *M*) the frog's stomach muscle loses all excitability, and may pass into a tonic contraction, presumably due to imbalance of ions without and within the muscle fibres. If about 10 per cent. of the sodium chloride of the saline is repalced with sucrose, then the mechanical response diminishes within a minute and becomes steady in 10 to 25 minutes. This shows that there is no diffusion barrier against sodium, such as might be due to a connective tissue sheath around the muscle fibres. Drugs, such as adrenaline, acetylcholine produce their effect almost immediately, showing that they have no particular difficulty in reaching the surface of the fibres.

If the osmotic pressure of the sucrose solution is reduced by half, so that its strength is 0·112 *M*, then the muscle behaves differently. When the muscle is immersed in hypertonic sucrose solution, a phasic contraction is produced at first. This is followed by a tonic contraction (Fig. 1), and the muscle does not respond to any stimulation. This period of depressed excitability usually lasts for about 2 hours at 25–30° C., and longer at lower temperatures. The response then increases in magnitude and may be several times bigger than that which occurs normally in saline. The muscle contracts rhythmically at 25–30° C. in the hypotonic solution (Figs. 1, 2). The response gradually declines, till in 24 hours it is about 20 per cent. of normal (Fig. 3). The response to alternating current in saline is bigger than that to direct current, but after some time in sucrose, reverse is the case,

so that later on, it may not respond to alternating current, but continues to respond to direct current. Acetylcholine causes contraction, and adrenaline relaxation.

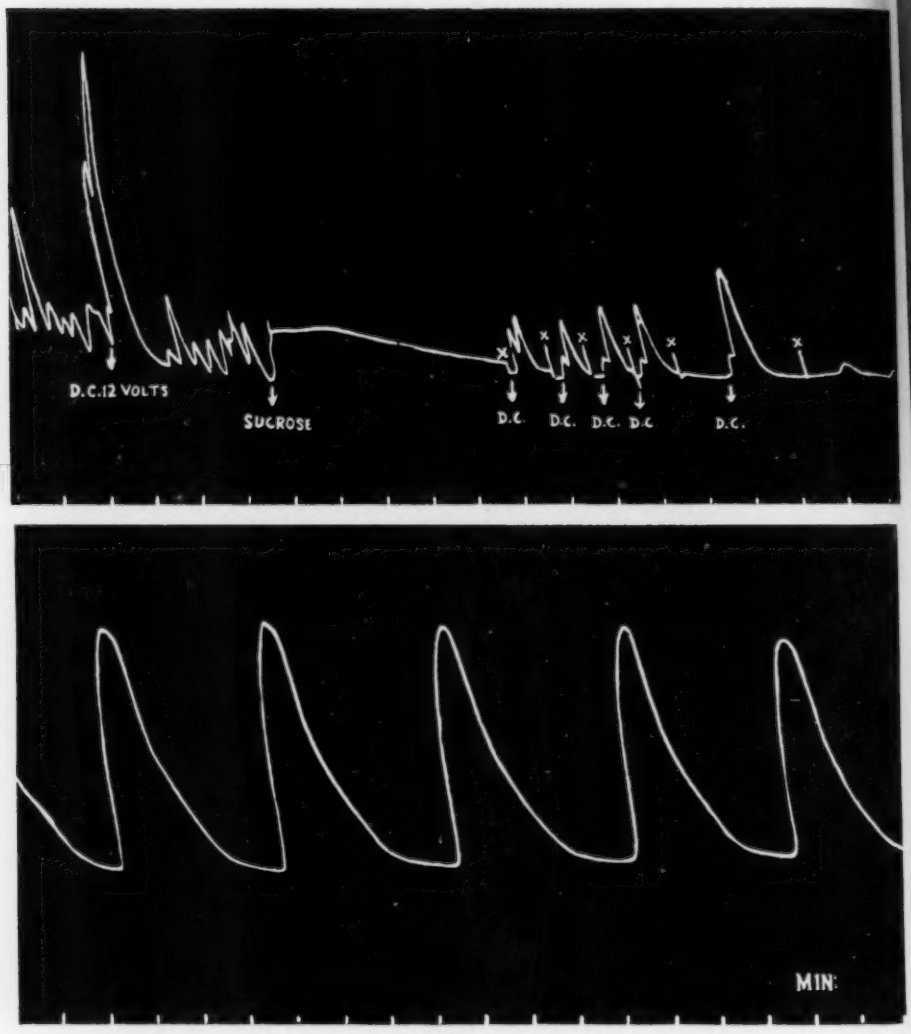

FIG. 1. Frog's stomach muscle. It was at first immersed in saline, then in hypotonic sucrose (0·112 *M*). At points marked D.C., it was stimulated with direct current, 12 volts for 10 sec. At X, the drum was stopped. Lower tracing shows rhythmic contractions in sucrose solution at the end of 3 hours. The sucrose solution was renewed every 15 minutes.

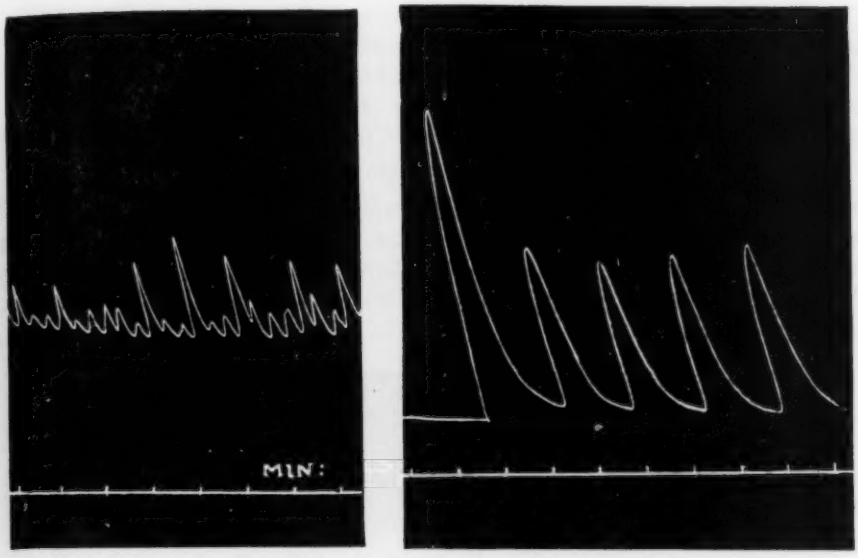

FIG. 2. Frog's stomach muscle. 1st tracing shows rhythmic contraction in saline. 2nd tracing shows rhythmic contractions 3 hours after hypotonic sucrose (0·112 *M*).

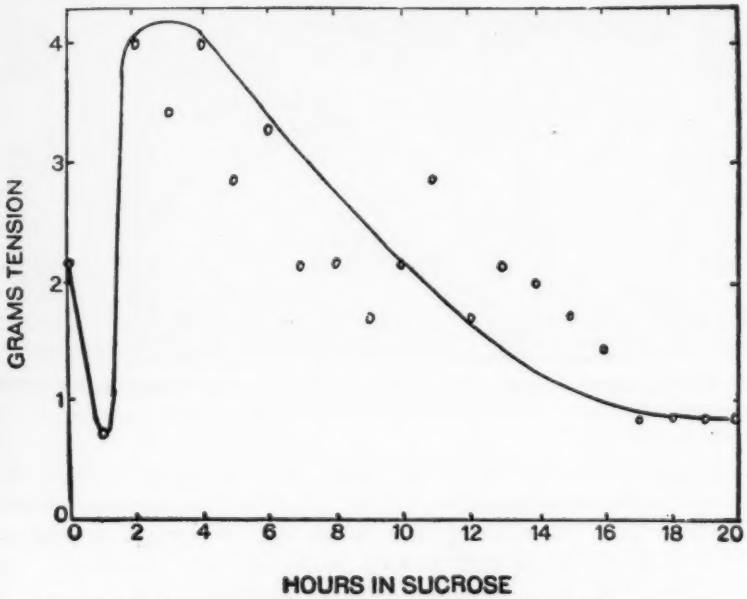

FIG. 3. Frog's stomach muscle. Effect of hypotonic (0·112 *M*) sucrose.

Spontaneous contractions are a companied by irregular electrical oscillations, suggesting asynchronous contraction of fibres (Fig. 4). When the muscle is contracting strongly, chemical analysis shows that it does not contain any significant amount of sodium. No precipitate is obtained with uranyl zinc acetate.

When the muscle is contracting strongly, or afterwards, reintroduction of sodium chloride causes immediate depression of excitability. The spontaneous contractions are abolished for about 2 hours, before they return to normal (Fig. 5). Thus the muscle at first acclimatises to sucrose. When it has thus acclimatised, sodium is found to be toxic. Similarly, it acclimatises to sodium.

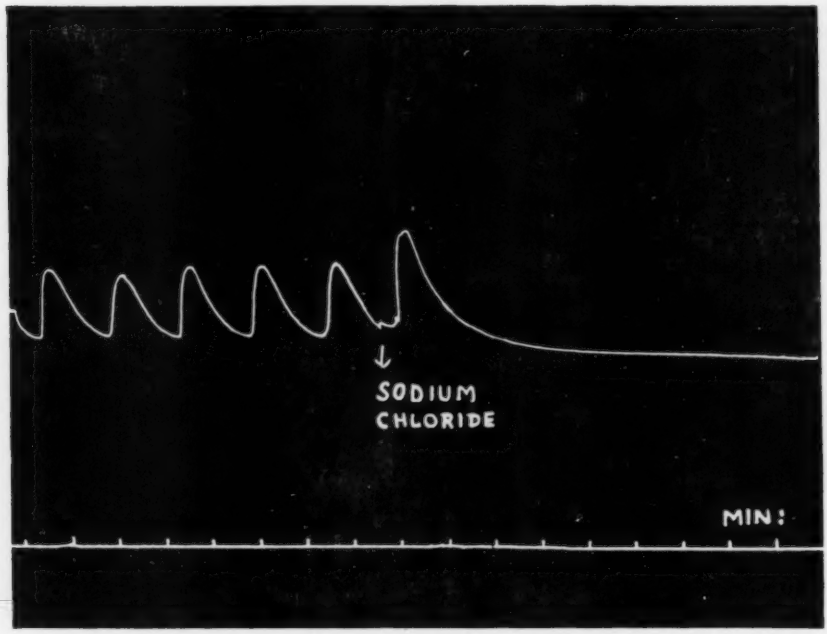

FIG. 5. Frog's stomach muscle. The muscle is contracting rhythmically in hypotonic (0·112 *M*) sucrose. Immersion in 0·112 *M* sodium chloride produces immediate depression of the mechanical response.

The measure of excitability of the muscle in these experiments is the height of spontaneous contractions, as the variation in the mechanical response due to electrical stimulation might be due to changes in conductivity of the solution containing varying quantities of electrolyte. In these experiments, the silver or silver chloride electrodes were placed at a

distance of 1 to 2 inches above and below the muscle in the muscle chamber and the muscle contracted when stimulated electrically even though the intervening sucrose solution was practically non-conducting. The sciatic nerve from the frog *Rana tigrina* can be similarly stimulated, though the electrodes may be placed in the sucrose solution laterally at a distance.

The dog's stomach muscle becomes inexcitable in hypotonic sucrose, but responds to electrical and chemical stimulation for about 12 hours if the temperature is kept from 18 to 20° C. The tænia coli of the guinea pig become inexcitable in about 10 min. The sartorius and gastrœnemins muscle from the frog *Rana tigrina* become inexcitable in an hour's time, but the sciatic nerve responds for over 12 hours when teased into smaller bundles. The heart from the same frog responds for about an hour both electrically and mechanically (Singh, Sehra and Singh, 1945 *a*, *b*). There is, however, great variability in its reaction to sucrose; some frog's hearts become inexcitable within 10 minutes in sucrose solution. The neuro-muscular junction however is more susceptible to sodium lack than either the nerve or muscle (Singh and Singh, 1948).

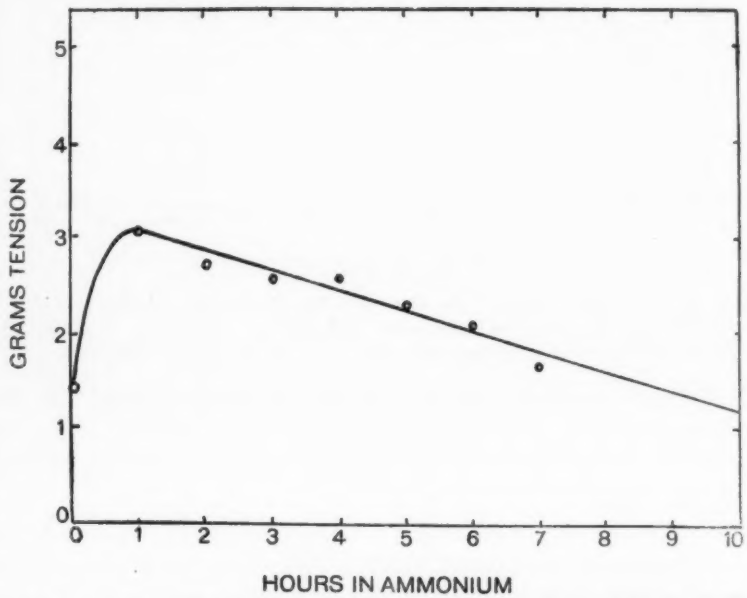

FIG. 6. Frog's stomach muscle. The effect of saline, the sodium chloride of which had been replaced with iso-osmotic ammonium chloride, on the mechanical response. Solution changed every 15–30 minutes. Muscle stimulated with D.C., 12 volts for 10 sec. each time.

Effect of other ions.—The frog's stomach muscle is remarkable in the fact that it gives apparently normal mechanical responses if the sodium of the saline is completely replaced with other ions, such as potassium, ammonium, calcium, magnesium and strontium (Singh and Singh, 1947). In ammonium, magnesium, calcium and strontium the responses are bigger than in saline (Figs. 6, 7, 8, 9), but in potassium chloride they are depressed, though the muscle responds for about 12 hours (Fig. 10). The sodium

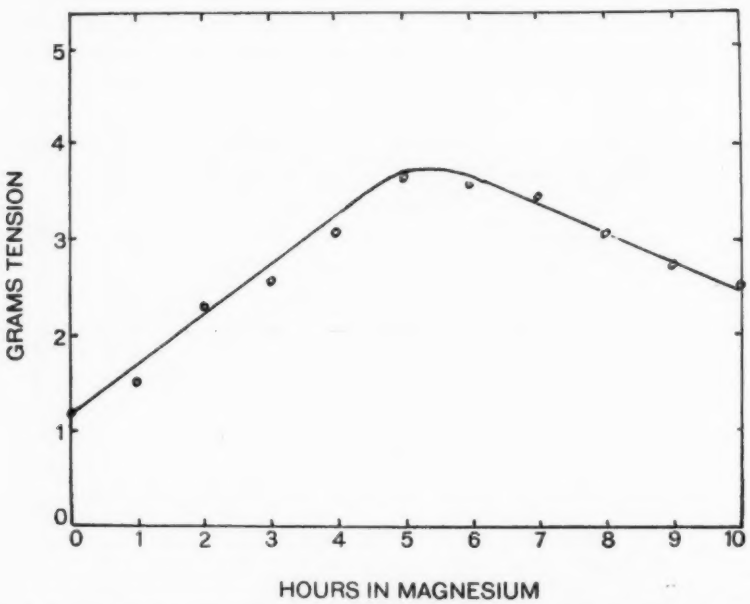

FIG. 7. Frog's stomach muscle. The effect of saline, the sodium chloride of which had been replaced with iso-osmotic magnesium chloride, on the mechanical response. Solution changed every 15–30 minutes. Muscle stimulated with D.C., 12 volts for 10 sec. each time.

content of these muscles is such that it does not give a precipitate with uranyl zinc acetate; it is thus negligible. The muscle shows spontaneous contractions in ammonium, calcium, magnesium and strontium chlorides (Fig. 11), but not in potassium chloride.

After the muscle has acclimatised to sucrose it becomes quite sensitive to small concentrations of calcium which are normally present in the saline. These small concentrations (0·003 *M* $CaCl_2$) produce a tonic contraction. Sodium chloride usually produces a twitch, but it may also produce a tonic contraction. Potassium chloride acts likewise.

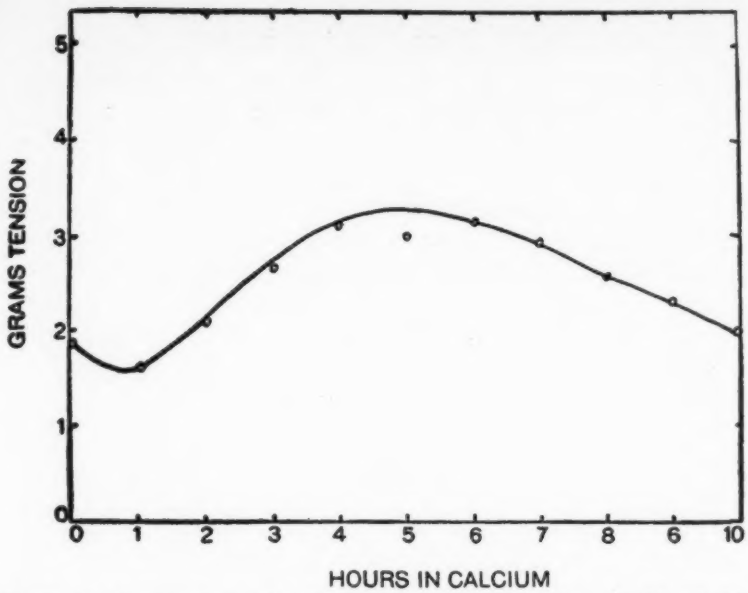

FIG. 8. Frog's stomach muscle. The effect of saline, the sodium chloride of which had been replaced with iso-osmotic calcium chloride, on the mechanical response. Solution changed every 15–30 minutes. Muscle stimulated with D.C., 12 volts for 10 sec. each time.

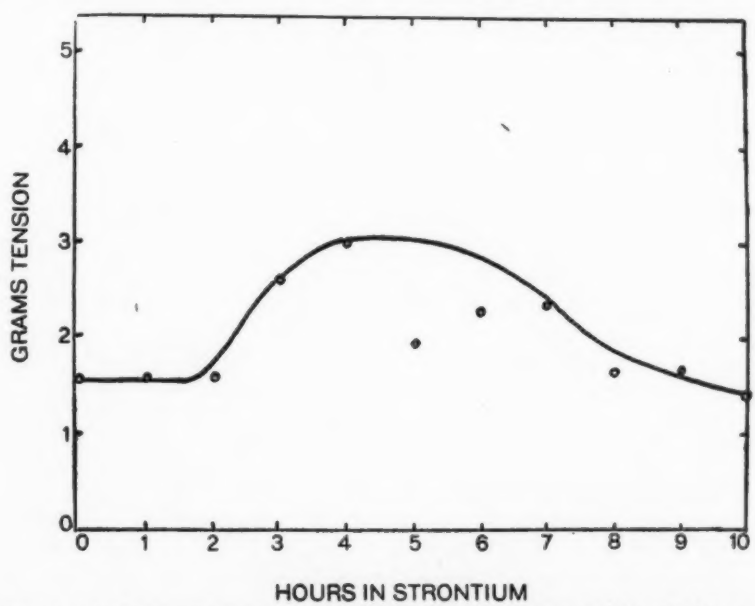

FIG. 9. Frog's stomach muscle. The effect of saline, the sodium chloride of which had been replaced with iso-osmotic strontium chloride, on the mechanical response. Solution changed every 15–30 minutes. Muscle stimulated with D.C., 12 volts for 10 sec. each time.

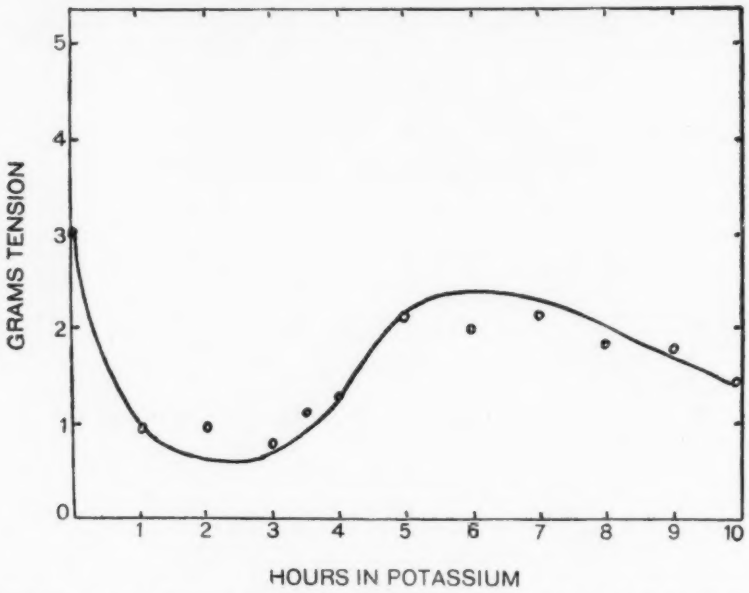

FIG. 10. Frog's stomach muscle. The effect of saline, the sodium of which had been replaced with potassium, on the mechanical response. Solution changed every 15–30 minutes. Muscle stimulated with D.C., 12 volts for 10 sec. each time.

When immersed in sucrose, the behaviour of frog's stomach muscle from different animals shows variations; muscles from some animals become inexcitable in 4 to 5 hours, whilst others remain excitable for over 24 hours. This variability is partly due presumably to loss of potassium, as the irritability of the muscles is maintained for longer periods, if a little potassium is added to the sucrose solution, the optimum concentration being 0·0036 *M* KCl. Even much smaller concentrations of potassium chloride, such as one-fifth of the above amount keep the muscles in a good excitable condition for several hours. Small concentrations of calcium chloride, normally present in the saline, do not exert any beneficial effect. On the contrary the duration during which the muscle remains excitable may become shorter. Calcium in the normal saline is therefore required to antagonise sodium. The gradual diminution of the response in sucrose as shown in Fig. 3, is partly due presumably to the loss of potassium, as shown by preliminary potassium estimations in the muscle; a full account of these estimations will be presented in a subsequent paper.

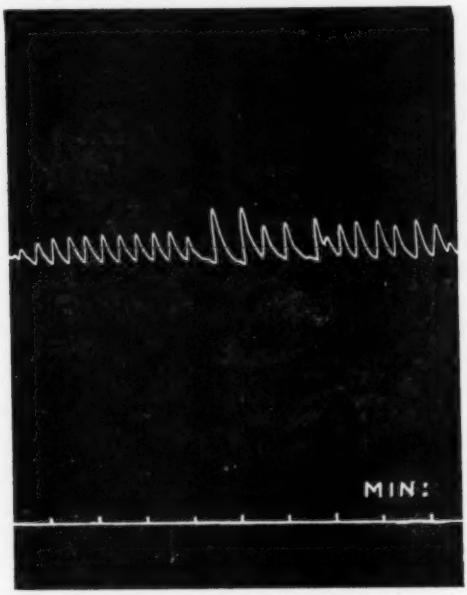

FIG. 11. Frog's stomach muscle. Spontaneous contraction in saline, the sodium chloride of which had been replaced with iso-osmotic strontium chloride, after 10 hours immersion and frequent renewal of the solution.

DISCUSSION

These experiments leave no doubt as to the dispensability of sodium for excitability and action potentials. The quantity of sodium present in the muscle soaked in sodium-free solutions must be utterly negligible. It may be argued, however, that the muscle responds owing to retention of sodium behind some connective tissue sheath which prevents its diffusion outwards. That this is not likely is shown by the fact that there appears to be no such diffusion barriers for sodium and other substances.

The crucial experiment which excludes the above possibility, is the one which demonstrates the depressant action of sodium after the muscle has acclimatised to sucrose (Fig. 4). As a matter of fact, more thoroughly the soduim is washed out, such as by changing the surrounding sucrose solution every 15 minutes, the better the muscle behaves. If the excitability of the muscle was due to retention of sodium in the interspaces between the fibres, such a depressant action of sodium could not occur. When the muscle has acclimatised to surcose, sodium becomes toxic.

The clue to the action of sodium in excitability is provided by the action of other ions. The frog's stomach muscle is unique in another way, that it responds in the presence of large concentrations of other ions such as ammonium, potassium, magnesium, calcium and strontium, which usually render other tissues rapidly inexcitable. This shows that the membrane of frog's stomach muscle fibres is comparatively resistant, and the action of sodium is to increase this resistance. In the membrane of frog's muscle, there must be some other factor present which allows sodium to be dispensed with. In tissues which become inexcitable in the absence of sodium, this factor must be deficient. The variability in the response of various tissues, or seasonal variations, are then probably due to the variable action of this second factor. The membrane of frog's stomach muscle is therefore comparatively resistant, and hence the muscle can be deprived of sodium, and still maintain its excitability.

Since the nature of excitability and the electrical response must have a common basis in all biological tissues, it appears to us that the role required of sodium in the hypothesis of Hodgkin and Katz (1945) is very unlikely.

When the muscle is immersed in full tonic sucrose solution there is a great imbalance of ions within and without the muscle fibres, hence the muscle becomes inexcitable. The recovery of excitability in half-tonic sucrose solution is due to the fact, that in a hypertonic solution, the entrance of water into the muscle fibres would reduce the concentration of ions within the fibres, and thus partly remove the ionic imbalance within and without the fibres. This view is supported by the fact, that if there is excess of potassium outside the fibres, its depressant effect is increased if the saline is rendered hypotonic, and decreased, if the saline is rendered hypertonic (Singh, 1939). Increase in tonicity of the saline would increase the concentration of potassium inside the fibres (Gokhale and Singh, 1945) and thus offset the increase of such concentration outside the fibres. Hypotonic solution would have an opposite effect. This view is supported by the fact that the depressant effect of increase in osmotic pressure of the saline is antagonised by increase in concentration of potassium outside the fibres (Singh, 1945).

SUMMARY AND CONCLUSIONS

1. If frog's stomach muscle is immersed in hypotonic sucrose solution, it maintains its excitability for about 24 hours, after a preliminary depres-

sion. The responses in the sucrose solutions may be several times bigger than in saline.

2. The spontaneous contractions of the muscle in sucrose solution are accompanied by action potentials.

3. When the muscle is contracting strongly in the sucrose solution, the sodium content (of the muscle) is negligible thus showing that sodium is not necessary for the production of mechanical and electrical changes.

4. When the muscle has acclimatised to sucrose, sodium has a depressant action, showing that the mechanical and electrical responses of the muscle are not due to any retention of sodium in the interspaces between the fibres.

5. The muscle also responds as in sucrose solution, if all the sodium of the saline is replaced with ammonium, potassium, calcium, strontium and magnesium. This suggests that the membrane of this tissue is comparatively resistant and hence this muscle can be deprived of sodium, and still maintain its excitability.

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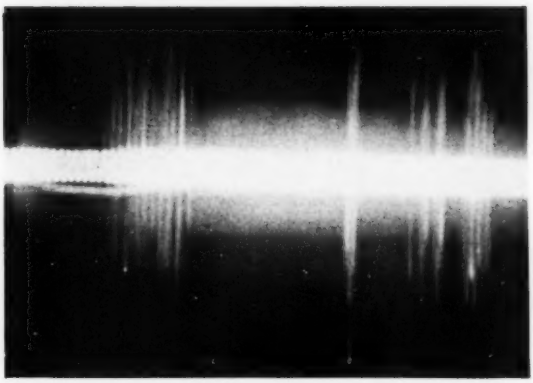

FIG. 4. Frog's stomach muscle. Action potentials due to spontaneous contractions after 3 hours immersion in hypotonic (0·112 *M*) sucrose.

PRELIMINARY OBSERVATIONS ON THE TOTAL PHOSPHORUS CONTENT OF THE INSHORE WATERS OF THE MALABAR COAST OFF CALICUT

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(Communicated by Dr. N. K. Panikkar, F.A.SC.)

Introduction

Although the occurrence and distribution of phosphates in sea-water have been the subject of extensive studies and its significance as a plant nutrient influencing seasonal cycles of plankton production well recognised, investigations on total phosphorus content have been found to be also useful in the light of recent advances concerning the phosphorus cycle in the sea. While the observed fluctuations in the levels of readily available nutrients in soluble inorganic forms may throw some light on the factors controlling productivity, it has been suggested in recent years that total phosphorus may serve as an index of the potential fertility of water masses in different regions. Apart from the amount usually estimated which exists as orthophosphate, phosphorus in sea-water is made up of various fractions both organic and inorganic in nature. A small proportion of the total is present in the standing crop of plants, in zooplankton and in organic detritus in suspension. Dissolved organic phosphorus compounds are also prominent in certain seasons and some turbid waters contain in addition inorganic particles like ferric phosphate. Kalle (1937) envisaged the probability that total phosphorus in dissolved compounds was a stable constituent of water masses which could serve as 'Hilfsmittel zur untersheidung von wasserkörpern'. Redfield and co-workers (1937) have made a detailed study of the phosphorus cycle in waters of the Gulf of Maine which indicated that while the proportions of different forms undergo seasonal variations, total phosphorus content of a limited region was relatively constant. These results were later confirmed by Armstrong and Harvey (1950) by studies on the waters of the English Channel. Since the west coast of India supports a rich fishery and the region is also characterised by a marked seasonal cycle in its biological features, the present study of the total phosphorus distribution of the area was undertaken as a part of comprehensive hydrobiological investigations in progress. A few observations made on the waters of the east coast are also included in the present account.

MATERIAL AND METHODS

Samples of sea-water have been collected once a week from a station located in the 8 fathom area located two miles off West Hill, Calicut (70° 46′ E & 11° 17′) employing Casella bottles for the collection of bottom water samples. Samples were also collected from the 20 fathom area about 10 miles away from the coast on many occasions as also from the Quilandy Bay, 7 miles north of West Hill. A few observations on the total phosphorus levels in the Korapuzha estuary, 4 miles north of West Hill, were also available during the same period for assessing the influence of river drainage on the inshore hydrological conditions. Total phosphorus was estimated by the method described by Hansen and Robinson (1953) and correction for the reagent blank was applied. Soluble inorganic phosphorus was estimated by the well known Deniges-Atkins method (Atkins, 1923) but no allowance has been made for salt error.

RESULTS

Since regular collections could not be made from the offshore station in the 20 fathom area, data from the station in the 8 fathom region alone have been recorded in the form of monthly average values in Table I. However, it may be mentioned that values from the offshore station were of

TABLE I

Monthly average values of total phosphorus and inorganic phosphate in sea-waters off West Hill during 1954–55 *in microgram-atoms per litre*

Month	Total Phosphorus		Inorganic Phosphate		Percentage of total in inorganic from	
	Surface	Bottom	Surface	Bottom	Surface	Bottom
April 1954 ..	1·73	2·37	0·64	0·80	37·0	33·8
May ..	2·34	6·82	0·49	1·16	20·9	17·0
June ..	1·15	1·85	0·59	1·76	51·3	95·1
July ..	3·94	9·36	0·95	2·17	24·1	23·2
August ..	9·46	10·43	1·08	1·76	11·4	16·9
September ..	5·73	6·61	1·92	3·06	33·5	46·3
October ..	5·40	3·44	0·80	0·71	14·8	20·6
November ..	4·68	4·69	0·53	0·85	11·3	18·1
December ..	3·90	4·87	0·33	0·58	8·5	11·9
January 1955 ..	5·66	4·10	0·53	0·66	9·4	16·1
February ..	6·58	6·38	0·67	0·77	10·2	12·1
March ..	6·77	6·87	0·65	1·00	9·6	14·6

the same order. Table II refers to the observations on the waters of the Palk Bay and Gulf of Mannar near Mandapam during the winter months of 1953. Graphical illustration of the seasonal variation is provided in

TABLE II

Monthly average values of total phosphorus and inorganic phosphate in the inshore waters of the Palk Bay and Gulf of Mannar during 1953 *to* 1954 *in microgram-atoms per litre*

Month	Total Phosphorus		Inorganic Phosphate		Percentage of total in inorganic form	
	Surface	Bottom	Surface	Bottom	Surface	Bottom
			Palk Bay			
September 1953	0·58	0·56	0·10	0·12	17·2	21·4
October ..	0·53	0·53	0·13	0·13	24·5	24·5
November ..	0·60	0·86	0·16	0·15	26·7	17·4
December ..	0·59	1·16	0·11	0·18	18·6	15·5
			Gulf of Mannar			
October 1953	0·56	0·44	0·12	0·13	21·3	29·5
November ..	0·89	0·48	0·12	0·12	13·5	25·0
December ..	0·77	1·64	0·21	0·19	21·3	11·6
January 1954 ..	0·72	2·15	0·13	0·15	18·1	7·0

Figs. 1 and 2. It was observed that the total phosphorus content of inshore waters off Calicut was quite high, the range for surface waters being 1·15 to 9·46 microgram atoms per litre the corresponding range for inorganic phosphate being only 0·33 to 1·92. Total phosphorus levels registered a sudden increase in July followed by a gradual lowering from September to December. The values again showed an upward trend thereafter till March 1955. Seasonal variation of the inorganic phosphate fraction was more uniform in nature with a well-defined peak in September. Bottom values were generally higher but the trends of variation were closely related to the changes in the surface layers. Inorganic phosphate constituted only

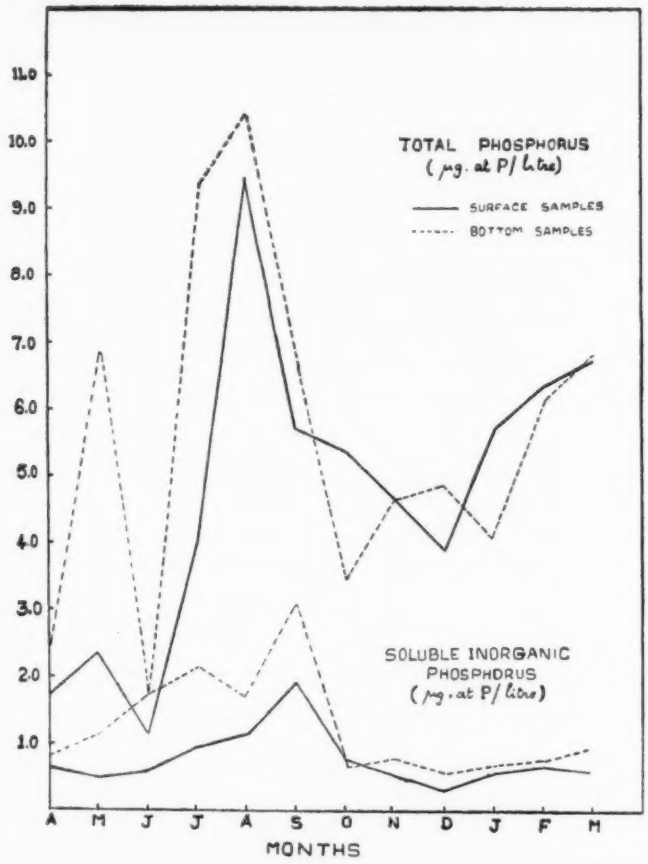

FIG. 1. Seasonal variations in total phosphorus and soluble inorganic phosphorus levels during 1954–55 in the inshore waters off West Hill, Calicut.

8·5 to 51·3% of the total throughout the year 1954–55. The same phenomenon was observed in the waters of the Palk Bay and Gulf of Mannar but the values recorded for total and inorganic phosphorus were much lower than in the Malabar waters.

DISCUSSION

For a proper understanding of the significance of the results of the present investigation, it would be necessary to consider the nature of the environment which is characterised by special climatological and topographical features. The Malabar Coast of India is subjected to a heavy rainfall

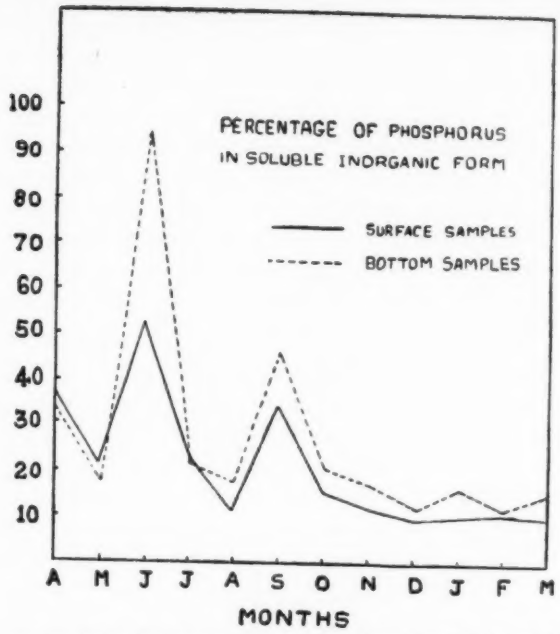

FIG. 2. Seasonal variation in the percentage of phosphorus present in soluble inorganic form in Calicut waters during 1954–55.

amounting to 120″ per annum and numerous streams carry enormous amounts of water so precipitated from the Western Ghats situated along the coast into the Arabian Sea. The sea bottom is predominantly muddy all along the west coast up to Mangalore with the presence of mud-banks at various places. The inshore waters in question are also influenced to a considerable extent by Korapuzha, Beypore and Kallayi rivers emptying within 10 miles of Calicut. Hydrological conditions off the Malabar Coast are primarily dependent on the S. W. monsoon which breaks out around June which results in severe agitation of the water column apart from the influx of fresh waters.

It may be observed from the data presented in the preceding section that the total phosphorus content of the inshore waters off the Malabar Coast is fairly high and is largely made up of the bound and organic forms. Inorganic phosphate levels in this region are known to be higher than the values reported from stations on the east coast (Jayaraman [a,b] 1953; 1954; George, 1953). The present study indicates that total quantity of phosphorus is much higher than the commonly estimated dissolved inorganic

component. Though the levels observed are greater than the values reported for the Gulf of Maine (0·45 to 1·15 μg.-ats./l.) and the waters of the English Channel (about 0·45 μg.-ats/l.) for the surface layers, the former refer to a region 30 miles away from the coast (250 m. deep). The latter concentrations were observed 5 miles off Plymouth. Investigations in the Gulf of Mexico along the Florida Coast in connection with the red tide phenomena showed that inshore waters, 3 miles off the coast contained only about 1·0 μg. at P per litre though the organic phosphorus fraction was generally more predominant. High concentrations of total phosphorus were observed to bear some relationship to local concentrations of *Trichodesmium*, (Graham *et al.*, 1954). Australian waters of the Bass Strait contain much lower quantities (0·09 to 0·36 μg.-ats. P/l.) according to Rochford (1956).

Redfield *et al.* (*loc. cit.*) observed that dissolved organic phosphorus compounds appear in large quantities in summer during the peak period of plankton production followed by an accumulation of inorganic phosphate in the winter months. The integral mean concentration of total phosphorus was practically unchanged being 9·4 to 11·0 gm. below one sq. metre. Their findings were confirmed by Armstrong and Harvey (*loc. cit.*) who attributed the changes observed in the total phosphorus content (integral mean) from 10 to 16 mgm. P/m.^{3} to the movements of water masses and their mixing.

Total phosphorus levels in the Malabar waters appear to vary within wide limits. Individual observations were far more variable than the monthly average values would indicate and ranged from 0 to 18·0 μg.-ats./l. for the surface waters. Monthly average values from the offshore station, 10 miles away from Calicut, showed a similar variation, 0·8 to 6·6 μg.-ats. in surface waters and 4·0 to 10·2 μg.-ats. in the bottom layers. The range for surface waters was 1·42 to 33·4 μg.-ats. in the Quilandy Bay. It does not, however, appear likely that rivers of this region contribute significant amounts of either organic or inorganic phosphorus compounds. Waters of the Korapuzha river were found to be poorer in both factors. However, the river mouth itself showed a higher concentration of total phosphorus presumably due to vertical mixing and replenishment from the bottom muds which were found to be rich in phosphates. The river itself is characterised by a sandy bottom.

Results of the present investigation indicate that the elevation of phosphate levels with the commencement of the monsoon is accompanied by more striking changes in the total phosphorus content. It is, however, possible that much of the total phosphorus, particularly in the monsoon

months, may represent phosphate liberated from colloidal soil phosphates by acid digestion and may not be directly available biologically (Rochford, 1951). A close study of the relative abundance of total and inorganic phosphate suggests that rise in total phosphorus precedes the replenishment of the inorganic phosphate levels and the changes are reflected earlier in the bottom waters in each case. All the three possible mechanisms suggested by Seshappa and Jayaraman (1956), namely release from the bottom muds, influx of silt-laden fresh-waters and large-scale mortality of bottom dwelling organisms at the commencement of the monsoon may account for the high concentrations of total phosphorus observed in the monsoon months. The last-mentioned probability appears to be more plausible and is of greater significance for the release of large quantities of dissolved organic phosphorus compounds in the bottom layers. However, it is not clear as to why the total phosphorus levels are relatively high during the post-monsoon months and the levels show an upward trend from December. Further work on the seasonal changes covering a longer period alone can give a fuller knowledge of the phosphorus cycle.

Apart from the usefulness of total phosphorus determinations for the identification of different water masses as adopted by Soule *et al.* (1954), a comparative study of the observations made at Mandapam with the data from the west coast confirms its value as an index of the fertility of different waters. The ease with which samples could be analysed after storage without noticeable deterioration and without the use of preservatives offers an additional advantage in oceanographical investigations as pointed out by Ketchum *et al.* (1955).

Summary

The object of this investigation was to study the total phosphorus content of the inshore waters with a view to understanding the phosphorus cycle in the sea. Seasonal variations in the total and soluble inorganic phosphorus concentration in the inshore waters of the Malabar Coast, a region highly productive from the fisheries point of view, was studied over a period of one year during 1954 to 1955 near West Hill, Calicut. Preliminary observations on the data collected have been presented in this account which also include some information on the total phosphorus content of the waters of the east coast near Mandapam. Sea-waters off Calicut were found to be rich in total phosphorus which varied from 1·2 to 9·5 μg.-ats. P/l. in the surface layers. Soluble inorganic phosphorus accounted for only 8·5 to 37·0% of the total except in the month of June when the onset of the S.W. monsoon is followed by a steep rise in the total as also the inorganic

phosphate. It is probable that release from the bottom muds as a result of the severe agitation of the water column in the monsoon months may explain the high levels of phosphorus compounds. Large-scale mortality of bottom dwelling animals may also play some part in this connection.

A comparative study of the levels on the east and west coasts confirms the usefulness of total phosphorus determinations as an index of the potential fertility of different regions.

ACKNOWLEDGEMENTS

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STUDIES ON INDIAN ICHNEUMONIDÆ (HYMENOPTERA)

Part I. Sub-Family PLECTISCINÆ

Part II. Sub-Family COLLYRIINÆ

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Introduction

In a previous paper* the importance of the parasite family of Ichneumonidæ (Hymenoptera) has been stressed from the point of view of its applied importance in the biological control of insect pests. No work has been done in India in the sub-family Plectiscinæ. Morley (1913) did not mention the sub-family Plectiscinæ at all. This sub-family has now been created according to recent trends in the taxonomic work of this family. All the available references to the various species have been verified and the recent views of the specialists on the synonymy of this group have been adopted.

Zoological Nomenclature

International rules on Zoological nomenclature as supplemented by opinions have formed the basis for this systematic work. All the super-generic names are based on the oldest included genus.

* Studies on Indian Ichneumonidæ, Sub-family Banchinæ Part I, Tribe Glyptini, *Proc. Ind. Acad. Sci.*, 1954, **40**, 38.

ACKNOWLEDGEMENT

Taxonomic work of this sub-family has been carried out in the Division of Entomology, Indian Agricultural Research Institute, New Delhi. We are indebted to Prof. Townes, author of *Catalogue and Reclassification of Nearctic Ichneumonidæ* for his valuable suggestions and comments. We wish to thank Dr. B. P. Pal, Director, Indian Agricultural Research Institute, for the keen interest he has taken in this research work.

ARRANGEMENT

Superspecific classification and ' Trivial names ' have been adopted from Muesebeck and others (1951) and described accordingly.

POSITION OF INDIAN PLECTISCINÆ

Ashmead (1900) placed this sub-family as tribe of Ophioninæ. But this is not new. *Plectiscus* Forst. was placed next before the genus *Porizon* by Marshal in his 1872 catalogue. Besides this Morley considered this sub-family only as a tribe of Ophioninæ and included *Megastylus* Schiödte as one of the genera in *Ichn. Brit.*, **5**: 3, (1913). But he placed *Megastylus* Schiödte, *Hyperacmus* Holm. and *Seticornuta* Morley in Tryphoninæ in *Fauna Brit. India, Hymen.*, **3**, (1913). Ashmead (1900) said, " This tribe, as at present defined, is scarcely a natural minor group since it comprises several discordant genera, with affinities allying them to genera in the Cryptinæ, the Tryphoninæ, and the Pimplinæ. Davis without stating his reasons for so doing, placed the group with Tryphoninæ. This is clearly an unnatural position for the majority of the genera." Even by placing this in Ophioninæ Ashmead said, " The true position of the tribe Plectiscini which as at present constituted is evidently an unnatural group, is still doubtful. It has affinities allying it with the Tryphoninæ, Cryptinæ and other of the sub-families." Cushman and Rohwar (1920) while dealing with Holarctic tribes of Ichneumon flies said, " The Plectiscini, Banchini and Paniscini are notable as bones of contention."

So far no work has been done on Indian Plectiscinæ. The genus *Megastylus* Schiödte placed in tribe Tryphonides and the genera *Hyperacmus* Holm. and *Seticormuta* Morley placed under tribe Exochides by Morley (1913) have been withdrawn on account of their different host relationship and adult characters. All these three genera have now been placed under this sub-family.

The members of this sub-family are recognised by the following characters:

Head usually small; clypeus always strongly discreted and invariably strongly convex with its sides generally compressed; front wing with stigma large broadly triangular, or broadly ovate; metathorax not produced into a neck at the apex; Spiracles small and circular; second recurrent vein joining the cubitus behind the transverse cubitus or interstitial with it; the cubital nerve in hind wing distinct; hind tibia a little incrassate and usually more or less constricted at the base; the tarsi always slender with claws simple not pectinate rarely large and curved.

We have almost no information on the economy of this sub-family. Fungivoridæ are believed to be its usual hosts as given by Muesebeck and others (1951). Its members are also said to prey exclusively upon Diptera mainly of the Nematocerous families Mycetophilidæ and Tipulidæ Morley (1913).

Systematically Arranged Catalogue of Indian Species

Hyperacmus Holmgren

Hyperacmus Holmgren, *Svenska. Vet. Akad. Handl.*, **1**: 322, (1855), (type of the genus: *Exochus crassicornis* Grav.). Dalla Torre, *Cat. Hymen.*, **3**: 222, (1901–02). Morley, *Fauna Brit. India, Hymen.*, **3**: 308, (1913).[1] Townes, *Mem. Amer. ent. Soc.*, **11** (2): 545, (1945). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agric. Monograph*, **2**: 345, (1951).

[1]Range. North & Central Europe, Himalayas, Connecticut.

Nothaima Cameron, *J. Bom. Nat. Hist. Soc.*, p. 428, (1902).

crassicornis (Gravenhorst)

Exochus crassicornis Gravenhorst, *Ich. Eur.*, **2**: 347, (1829).

[1]Punjab: Simla, United Provinces: Mussoori, Europe.

Hyperacmus crassicornis Holmgren., *Sevenska. Vet. Akad. Handl.*, **1**: 322, (1855). Brischke, *Schr. Phys. öken. Ges. Königsb.* p. 410, (1871): *Schr. Nat. Ges. Danz.*, p. 108, (1878). Thomson, *Deut. Ent. Zeit.*, p. 199, (1887). Dalla Torre, *Cat. Hymen.*, **3**: 222, (1901–02). Morley, *Fauna Brit. India, Hymen.*, **3**: 309, (1913), ♂, ♀[1]. Townes, *Mem. Amer. ent. Soc.*, **11** (1), 545, (1945).

Seticornuta Morley

Seticornuta Morley, *Fauna Brit. India, Hymen.*, **3**: 310, (1913),[1] (type of genus: *S. albicalcar* Morley).

[1]Range. Burma & Ceylon.

albicalcar Morley

Seticornuta albicalcar Morley *Fauna Brit. India, Hymen.*, **3**: 310, (1913). ♀[1].

[1]Tenasserim: Mergui; Ceylon: Kandy.

Megastylus Schiödte

Megastylus Schiödte, *Rev. Zool.* (*Soc. Cuv.*), **1**: 139, (1838); *Guer. Mug. Zool. Ins.*, pl. 6, (1839), (type of genus: *M. Cruentator* Schiödte). Dalla Torre, *Cat. Hymen.*, **3**: 15, (1901–1902). Morley, *Ich. Brit.*, **5**: 17, (1913); *Fauna Brit. India, Hymen.*, **3**: 341, (1913).[1] Townes, *Mem. Amer. ent. Soc.*, **11** (2): 540, (1945). Muesebeck, Krombein, Townes and others, *U.S. Dept. Agric. Monograph*, **2**: 343, (1951).

[1]Range. Palæarctic & Nearctic regions.

Brephoctonus Forster, *Verh. Naturh Ver. Preuss. Rheinl.*, **25**: 159, (1868).

longicoxis (Cameron)

Letosha longicoxis Cameron, *J. Bom. Nat. Hist. Soc.*, p. 785, (1909). ♀ as ♂.

Megastylus longicoxis Morley, *Fauna Brit. India, Hymen.*, **3**: 341, (1913). ♂[1].

[1]Punjab: Simla.

Sub-Family **COLLYRIINÆ**

Collyria Schiödte put under the tribe Acænitini of the sub-family Pimiplinæ by Morley (1913) in *Fauna Brit. India, Hymen.*, **3**, was found to be an anomalous genus of uncertain affinities. It is not very correct with our recent knowledge in taxonomic research to place it in Acænitini as has been done by Morley (1913). This subfamily was put on record for the first time in India which contains the only genus *Collyria* Schiödte.

Collyriinæ Muesebeck, Krombein, Townes and others, *U.S. Dept. Agric. Monograph*, **2**: 341, (1951).

Collyria Schiödte

Pachymerus Gravenhorst, *Ich. Eur.*, **3**: 721, (1929) Preocc.

Collyria Schiödte, *Mag. Zool.*, (Ser. 2) **1**: 10, pl. 6–10 (1839), (type of genus: *Pachymerus calcitrator* Grav.). Morley, *Ich. Brit.*, **3**: 261, (1907); *Fauna Brit. India, Hymen.*, **3**: 45, (1913).[1] Muesebeck, Krombein, Townes and others, *U.S. Dept. Agric. Monograph*, **2**, 341, (1951).

[1]Range. Europe, North Africa, China & Ceylon.

gracilipes (Mots.)

Pachymerus gracilipes Motschulsky, *Bull. Soc. Imp. Nat. Moscon*, p. 30, (1863).

Collyria gracilipes Morley, *Fauna Brit. India, Hymen.*, **3**: 47, (1913).[1]

[1]Ceylon: summit of Mount Patannos.

unifasciatus (Mot.)

Pachymerus unifasciatus Motschulsky, *Bull. Soc. Imp. Nat. Moscon*, p. 30, (1863).

Collyria unifasciatus Morley, *Fauna Brit. India, Hymen.*, **3**: 46, (1913).[1]

[1]Ceylon: Nuwara Eliya.

IN VIVO DETECTION OF GIBBERELLIC ACID IN 'FOOT-ROT' INFECTED RICE (*ORYZA SATIVA* L.)

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Received January 15, 1957

(Communicated by Professor T. S. Sadasivan, F.A.SC.)

Gibberellic acid, a metabolic product of *Gibberella fujikuroi* (Saw.) Wr. (*Fusarium moniliforme* Sheld.), was isolated by Curtis and Cross (1954) from *in vitro* cultures of the fungus and found to induce the typical elongation of the stem of rice plants (Bakanæ effect), the characteristic natural symptom of 'Foot-rot' infected rice, induced by the fungus. Although pure gibberellic acid or neat culture filtrate of the pathogen could successfully reproduce the 'Bakanæ effect' when treated to fresh healthy rice plants, the *in vivo* detection of gibberellic acid has not been accomplished, a criterion so essential to establish the role of this growth-promoting substance in the disease syndrome. The present report is an outcome of an investigation undertaken to elucidate this problem.

Materials and Methods

Susceptible variety of rice plants, MTU 9, (*Oryza sativa* L.), were grown in sterilized, *F. moniliforme* infested and uninfested soils. Representative samples of roots and shoots of plants showing the typical 'Bakanæ' symptoms and healthy control plants grown in uninfested soils were washed with several changes of glass-distilled water, ground in ether and the supernatant made up to known volumes and used for chromatographic analysis. A sample of pure gibberellic acid was kindly supplied by Dr. P. W. Brian of the I.C.I. Ltd., Akers Research Laboratories, the Frythe, Welwyn, Herts. The concentrated ether extracts of plants and an alcoholic solution of pure gibberellic acid were chromatographed on Whatman No. 1 filter paper (7·4 cm. diameter), employing the circular paper chromatographic technique developed in this laboratory (Lakshminarayanan, 1954). The chromatograms were developed in five different solvent mixtures, air-dried and dipped in a saturated solution of silver nitrate, followed by an exposure to ammonia vapour. Sharp black bands against a brown background were noticed, indicating the position of gibberellic acid, which were intensified by keeping the chromatograms at 60° C. for three minutes. Quantitative determination of gibberellic acid was made by spotting known concentrations of the pure substance in the usual manner and developing the chromatograms in *n*-Butanol/Acetic acid/Water (4: 1: 5). The area of the band

formed after treating the chromatograms with detecting agents was calculated. The average value of six replicates for each concentration was used to draw the concentration-area graph.

RESULTS

Table I presents the R_f values of *in vivo* gibberellic acid in roots and shoots of rice plants showing 'Bakanæ' symptoms. The R_f values in all the five different solvent mixtures closely correspond with that of pu

TABLE I

R_f-values of pure and in vivo *gibberellic acids in five different solvent mixtures*

Material	R_f values				
	n-Butanol/ Acetic acid/ Water (4:1:5)	Phenol/ Water (1:1)	*n*-Butanol/ Formic acid/ Water (8:2:10)	*n*-Butanol/ Water (1:1)	Ethyl acetate/ Pyridine/ Water (2·5:1:3·5)
1. Pure gibberellic acid ..	0·404	0·216	0·188	0·188	0·566
2. 'Bakanæ' roots..	0·416	0·200	0·188	0·188	0·576
3. 'Bakanæ' shoots	0·404	0·212	0·196	0·188	0·560
4. Healthy roots	No bands				
5. Healthy shoots					

gibberellic acid. The extracts of healthy plants did not reveal any bands, indicating the absence of gibberellic acid in them. The *in vivo* nature of gibberellic acid was further substantiated by developing the chromatograms in *n*-Butanol/Acetic acid/Water (4:1:5), eluting them with ether at the specific R_f and testing the eluate for gibberellic acid activity. While the eluate from the infected plants reproduced the 'Bakanæ effect' on fresh healthy rice seedlings, the eluate from healthy plants did not. Plate IX presents the circular paper chromatograms of pure and *in vivo* gibberellic acids of roots and shoots of infected rice. Fig. 1 shows the concentration of gibberellic acid and area of bands, bearing a linear relationship. Using this standard graph the quantity of *in vivo* gibberellic acid in roots and shoots

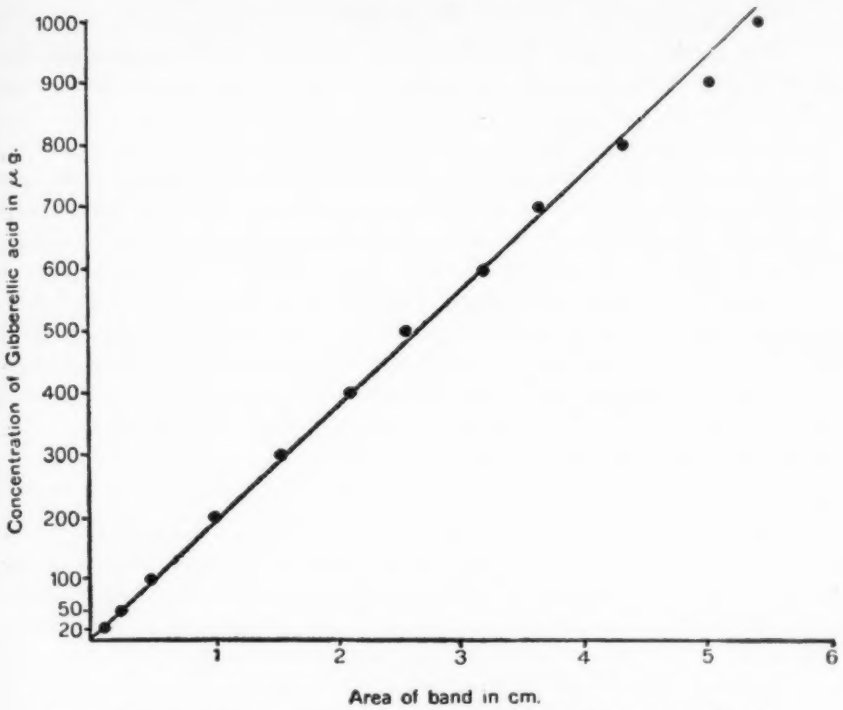

FIG. 1. Graph showing the concentration of gibberellic acid and the area of bands.

of infected rice plants was calculated and is presented in Table II. The results indicate a greater accumulation of gibberellic acid in shoots than roots.

TABLE II

Quantity of in vivo *gibberellic acid present in roots and shoots of* F. moniliforme *infected rice plants*

	Material	Quantity of *in vivo* gibberellic acid (mg./100 g. fresh weight)
1.	'Bakanæ' roots ..	2·80
2.	'Bakanæ' shoots ..	5·55
3.	Healthy roots ..	0·0
4.	Healthy shoots ..	0·0

DISCUSSION

The modern trends in toxicological research have necessitated the detection of the components of culture filtrates of the pathogens in the infected hosts, a point of considerable importance in ascribing them a role in disease production (Dimond and Waggoner, 1953; Subramanian, 1955). Increasing evidences obtained in this laboratory by chromatographic and bioassay techniques have demonstrated the vivotoxic nature of fusaric acid in the case of fusariose wilt of cotton (Lakshminarayanan and Subramanian, 1955; Kalyanasundaram and Venkata Ram, 1956). The present finding is yet another evidence regarding the role of microbial products as *in vivo* causative agents of plant disease. Indeed, the demonstration of gibberellic acid *in vivo* within infected rice plants and the ability of such *in vivo* gibberellic acid to induce elongation of naturally infected host plants is not only indicative of its role in disease production but also an evidence in favour of regarding it as a true auxin.

SUMMARY

A chromatographic method for qualitative and quantitative determination of gibberellic acid in *F. moniliforme* infected rice plants has been described. The concentration of gibberellic acid and the area of the band on a circular paper chromatogram bear a linear relationship. The results indicate a greater accumulation of gibberellic acid in shoots than roots of the rice plant var. MTU 9, susceptible to the fungus.

ACKNOWLEDGEMENTS

The author is grateful to Professor Dr. T. S. Sadasivan for guidance and Dr. C. V. Subramanian for critically going through the manuscript. Thanks are due to Dr. P. W. Brian of the I.C.I. Ltd., Akers Research Laboratories, the Frythe, Welwyn, Herts., for a gift sample of gibberellic acid.

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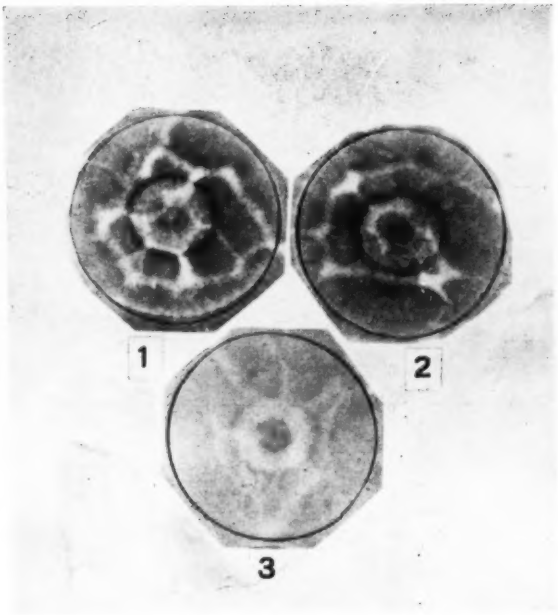
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CONTRIBUTION TO OUR KNOWLEDGE OF THE PHYSIOLOGICAL ANATOMY OF SOME INDIAN HYDROPHYTES

V. The Stem of *Lindernia* Allioni

BY M. V. MIRASHI, M.SC.

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Received December 4, 1956

(Communicated by Dr. T. S. Sabnis, F.A.SC.)

INTRODUCTION

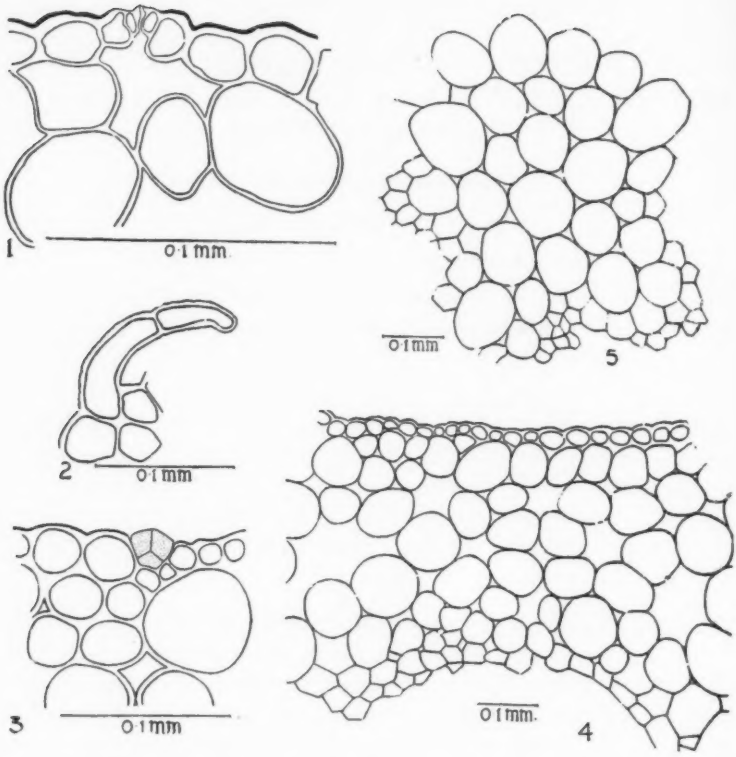
THE genus *Lindernia* Allioni of the Scrophulariaceæ was reconstituted by Pennell (1935) after merging with it the genera *Vandellia* Brown ex. Linn., *Ilysanthes* Raf. and *Bonnaya* Link. & Otto. The total number of species under *Lindernia* Allioni, in its new and amplified form, would be about seventy. Mukerjee (1945) enumerates twenty-five species of this genus occurring in India.

Lindernia Allioni is a typical hydrophytic genus whose species are mostly annual herbs that thrive usually in marshy areas, rice fields and damp places. In the present contribution it is proposed to give an account of the physiological anatomy of the stem in four species occurring at Nagpur, *viz.*, *L. crustacea* (Linn.) F. Muell., *L. hirsuta* (Bth.) Wettst., *L. ciliata* (Colsm.) Pennell, and *L. parviflora* (Roxb.) Haines.

The stem in all the species is erect or sub-erect and often diffusely branched. In *Lindernia parviflora* (Roxb.) Haines the stem is particularly slender and glabrous. *L. crustacea* (Linn.) F. Muell. and *L. ciliata* (Colsm.) Pennell have sparsely hairy stems. In *L. hirsuta* (Bth.) Wettst. the stem is succulent and clothed with many spreading hairs.

ANATOMY

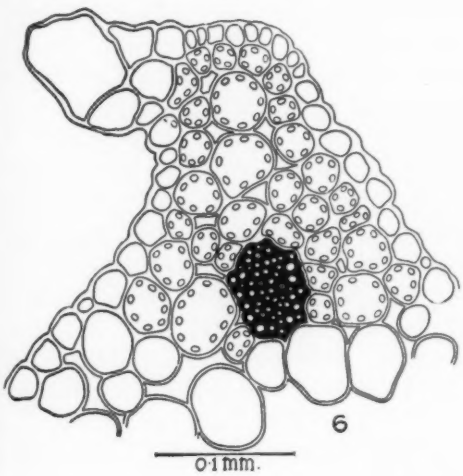
1. *Epidermis.*—Outer walls thicker than the undulated lateral walls; cuticle very thin, though recognizable, in all the four species (Fig. 1); anthocyanin present characteristically in the epidermal cells of the stem of *L. hirsuta* (Bth.) Wettst. Unicellular or uniseriate bicellular, thick-walled, papillose trichomes present in the hairy stems (Fig. 2). Multicellular peltate glands in all the species (Fig. 3). Stomata raised; guard-cells smaller than the epidermal cells (Fig. 1).

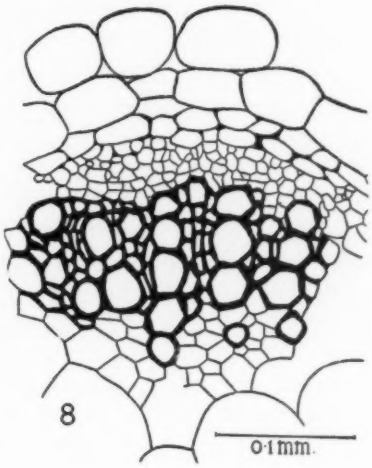


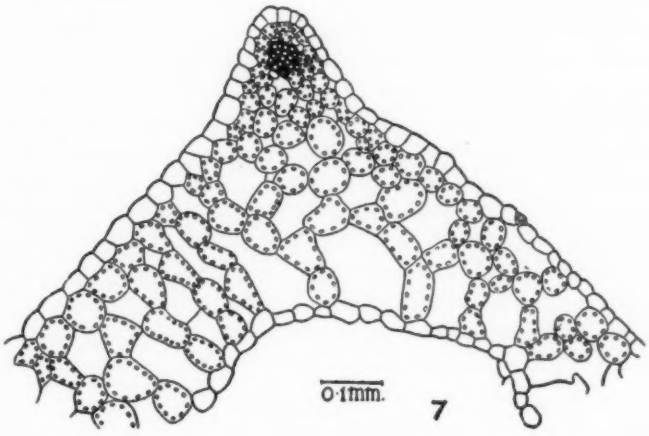
TEXT-FIGS. 1–5. *Lindernia hirsuta* (Bth.) Wettst. Fig. 1. Part of the T.S. of the stem showing the raised stoma, the small-sized guard-cells, the thin cuticle and thick outer walls of the epidermal cells. *Lindernia crustacea* (Linn.) F. Muell. Fig. 2. Part of the T.S. of the stem showing the uniseriate, bicellular thick-walled trichome. Fig. 3. Part of the T.S. of the stem showing the peltate gland in section. Fig. 4. Part of the T.S. of the stem showing the lacunar cortex. Fig. 5. Part of the T.S. of the stem showing the thin-walled parenchymatous pith cells with small intercellular spaces.

2. *Cortex.*—Collenchyma almost entirely wanting, only the outermost layer of the cortex showing collenchymatous differentiation (Fig. 6). Strands of sclerenchymatous fibres present in the sub-epidermal region of the ridges in the quadrangular outline of the stems (Figs. 6 & 7). Chlorenchyma usually confined to the cortical region of the ridge (Fig. 6), but in *L. parviflora* (Roxb.) Haines the entire cortex is chlorenchymatous. Intercellular lacunæ present in the cortex of all the species (Fig. 4). The cortical cells are generally spherical, thin-walled and the intercellular spaces roundish. The cortex of *L. parviflora* (Roxb.) Haines shows thin-

walled, elongated, chlorophyllose cells and prominently developed elongated lacunæ (Fig. 7). Endodermis is conspicuous.







TEXT-FIGS. 6–8. *Lindernia hirsuta* (Bth.) Wettst. Fig. 6. Part of the T.S. of the stem showing the chlorenchymatous cortex and the sub-epidermal sclerenchymatous rib in the ridge. Fig. 8. Part of the T.S. of the stem showing the endodermis, pericycle and the vascular bundle. *Lindernia parviflora* (Roxb.) Haines. Fig. 7. Part of the T.S. of the stem showing the profusely lacunar and entirely chlorenchymatous cortex and the more superficially situated sclerenchymatous rib.

3. *Pericycle.*—1–2 layered, walls slightly thickened (Fig. 8).

4. *Vascular system.*—Vascular bundles separated by prosenchymatous cells, typical parenchymatous medullary rays absent. Xylem and phloem well developed (Fig. 8).

5. *Pith.*—Thin-walled parenchymatous, intercellular spaces small (Fig. 5).

DISCUSSION

1. Scrophulariaceæ is one of those advanced families of Angiosperms which, although thriving mainly on land, include a number of hydrophytic forms. The genera *Hydrotirche* Zucc., *Limnophila* R. Br., *Dopatrium* Buch-Ham. ex. Benth., *Veronica* Linn., *Bacopa* Aubl. and *Lindernia* Allioni exhibit various degrees of adaptation to the aquatic conditions in their morphological as well as anatomical characters.

2. The foregoing account of the physiological anatomy of the stem of the four species of *Lindernia* Allioni, that thrive in damp places or swampy areas, brings out clearly the anatomical adaptations of these species to their habitat. The various features enumerated above may be briefly reviewed as follows:

(*i*) *Aerating system.*—As in the case of most hydrophytes, the stems of the species investigated above also show a characteristic development of aerating tissue in the form of the lacunar cortex. Solereder (1908) has mentioned the occurrence of a lacunar cortex in the stem of another species, *Lindernia pyxidaria* All. Syeedud-din (1940) describes the lacunar cortex in the stem of another hydrophytic member of the Scrophulariaceæ growing in India, *Bacopa monnieri* (Linn.) Pennell (*Herpestis monniera* H. B. & K.). Of the four species investigated here, *Lindernia parviflora* (Roxb.) Haines exhibits a greater degree of adaptation in the form of a more fully developed cortical aerating system which consists of radially elongated, thin-walled, chlorophyllose cells separated by prominent schizogenous air-passages.

(*ii*) *Assimilatory system.*—The assimilatory tissue comprises the chlorenchymatous cortex which is generally confined to the ridges. Here, again, *L. parviflora* (Roxb.) Haines shows an advance inasmuch as the entire cortex is here collenchymatous and thus constitutes a prominent assimilatory region of the stem.

(*iii*) *Mechanical system.*—The disposition of the mechanical tissues in the stem of *Lindernia* Allioni deserves some special consideration. The thick-walled epidermis and the conspicuous endodermis provide concentric hollow cylinders of mechanical tissue for the stem. The sub-epidermal

sclerenchymatous ribs, situated in the projecting portions of the stem, recalling the pattern of their distribution in the stems of the Labiatæ, confer further rigidity on the organ. These are more superficially situated in the stem of *Lindernia parviflora* (Roxb.) Haines (Fig. 7) than in the stems of the other three species (Fig. 6) in which they have been reported earlier by Solereder (1908). The wood fibres associated with xylem vessels are well developed and they form the innermost ring of mechanical elements.

In the stems of most hydrophytes, the cortex is penetrated by lacunæ and is consequently weakened. One of the features, usually met with in the arrangement of the cortical cells of such stems, that seem in some measure to obviate the dangers of this fragility is that the outer cortex forms a firmer peripheral shell of collenchyma. Enough proof of this has been obtained in the stems of the composites investigated earlier by the author (Mirashi, 1954, 1955, 1956). It was only in the stem of *Cleome chelidonii* Linn. (Mirashi, 1956), which develops a lacunar pith in place of the usual lacunar cortex, that the characteristic collenchymatous hypodermis was not met with.

It is noteworthy, therefore, that in all the stems investigated here, and especially in the stem of *L. parviflora* (Roxb.) Haines, the collenchymatous outer cortex is conspicuous by its absence despite the fragile texture of the middle cortex which is lacunar. Now, the absence of a well-developed collenchymatous outer cortex in the stem is characteristic of the entire family Scrophulariaceæ. Here is an instance, then, of how the adaptive responses of plants to their environment are primarily conditioned by their inheritance. The mechanical requirements of the stem could be satisfied only within the limitations of its inherent characters. The role played by inherent ancestral tendencies needs proper emphasis while interpreting the adaptations of plants in general and the hydrophytes in particular. It is possible that the different phylogenitic histories of the hydrophytes may have been responsible for the wide variety of adaptations exhibited by them in spite of their relatively uniform environment as compared with that of the terrestrial plants.

Acknowledgement

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CHEMICAL STUDIES ON INDIAN SEAWEEDS

III. Partition of Sulphur and its Relation to the Carbohydrate Content

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Introduction

Seaweeds in general derive their importance from their carbohydrate content. Part of the carbohydrate is in the form of cellulose which cannot be hydrolysed by ordinary means; and the rest in the form of polysaccharides either as agar or as algin, the latter being considered as a polymerised form of *d*-mannuronic acid. Kylin (1913) is of the opinion that simple reducing sugars constitute the first products of photosynthesis and that they occur in very small quantities. Haas and Hill (1929) have found a pentose in *Pelvetia canaliculata* though it could not be detected in *Fucus serratus* and *Ascophyllum nodusum*. Recently much work has been done on the fucoidin content—a polysaccharide sulphate ester, of Phæophyceæ (Percival and Ross, 1950). The isolation of a polysaccharide, xylan, which gives only xylose on hydrolysis from the red seaweed, *Rhodymenia palmata*, has been reported by Percival and Chanda (1950). Dextrine like polysaccharides such as laminarin are a common feature of the Phæophyceæ while mannitol is said to be universal in them. In red seaweeds, on the other hand, mannitol is absent while other sugar alcohols like dulcitol and sorbitol have been detected (Haas and Hill, 1931 & 1932; Hassid, 1933).

The earlier work also indicate the importance of sulphur in the carbohydrate metabolism of the seaweeds. The composition of agar, the chief polysaccharide constituent of red seaweeds, as given by Jones and Peat (1942), itself indicates that SO_4 forms an integral part of the agar molecule. Barry and Dillon (1944) assume that there are as many as 53 galactose units to each SO_4 group with at least 140 such units to each non-reducing end group. Percival and Ross (1950) found a fixed percentage of sulphate in the polysaccharides isolated from a few species of brown seaweeds, *viz.*, *Fucus vesciculosus*, *F. spiralis*, *L. cloustoni* and *H. lorea*. Ross (1953) in his analysis of the red seaweeds of Great Britain observed wide variations in the sulphate content of the various species, the minimum figure being associated with a low galactose content or with none. The sulphate increases to a figure representing approximately one sulphate group per

hexose unit. It was further noticed that species containing any appreciable amount contained galactose as the primary sugar showing that these two are interrelated.

Takagi and Susuki (1952) have shown that sulphur containing amino acids are present in the seaweeds investigated by them. The presence of cystine—a sulphur containing amino acid—was recognised in all marine algæ which they studied—the quantity being largest in brown algæ, next in greens and least in reds.

In the present communication results of an attempt to study the partition of sulphur in eleven common seaweeds of the Indian coast in relation to their polysaccharide content and distribution are presented.

The species studied, the details of collection and the method of preparation of the sample for analyses have been dealt with in an earlier paper (Krishna Pillai, 1956).

METHODS OF ANALYSES

Total sulphur.—The method of Aitken (1930) was employed in the estimation of total sulphur in the seaweeds, Benedict-Denis reagent being used and the sulphur precipitated as $BaSO_4$. Dried seaweed samples, not more than 0·5 g. for each estimation, are accurately weighed and used.

Total sulphate.—One gram of the air-dried material was ashed carefully in a silica crucible without allowing the material to melt. After cooling the ash was taken up with water and filtered. The solution was heated to boiling point and sulphate was precipitated as barium sulphate with boiling barium chloride.

Ionic sulphate.—One gram each of the sample was taken and powdered with acid-washed sand. The material was taken up with glass distilled water and stirred continuously for an hour with a stirrer. The mixture was centrifuged and the clear extract used for the precipitation of sulphate.

Invert sugar.—One gram of the air-dried sample was macerated with glass distilled water and transferred to a beaker. The contents were stirred continuously for sometime and the resultant mixture centrifuged. To the clear solution was added 5 c.c. of 38% hydrochloric acid and the flask was placed in a water-bath maintained at 70° C. The solution was kept at 69° C. for 8 minutes. After cooling quickly the solution was neutralised with sodium hydroxide and treated with Fehling's solution. The Fehling's solution was prepared by mixing 5 c.c. of copper sulphate and 5 c.c. of alkaline tartrate solution. The mixture was diluted to about 50 c.c. and

heated in a regulated flame so that boiling began in four minutes. The boiling was continued for three more minutes and the precipitated copper oxide filtered through a weighed scintered crucible lined on the inside with asbestos mat which was previously treated. The precipitate that collected on the asbestos was washed with 95% alcohol three times followed by three washings with ether. The washed precipitate was dried at 105° C. for 2 hours and weighed.

Other cold water-soluble polysaccharides.—The sample was treated as before for the estimation of invert sugar. After removal of the water-soluble proteins by precipitation the extract was hydrolysed with 6N hydrochloric acid for 4 hours. After hydrolysis the solution was cooled, neutralised with sodium hydroxide and filtered. The filtrate was treated with 10 c.c. of Fehling's solution as indicated above and boiled. The precipitated copper oxide was filtered, washed and weighed.

Hot water-soluble polysaccharides.—One gram of the sample was ground well with water and heated for four hours on a boiling water-bath. This was filtered hot and the residue was extracted again. The clear extracts are combined and hydrolysed with 6N hydrochloric acid. As before the reducing sugars are estimated on aliquotes of the hydrolysates.

Total carbohydrate.—One gram of dried sample was ground well with acid-washed sand and the entire mixture was taken up with distilled water, and transferred to the apparatus for hydrolysis. The requisite quantity of acid was added and the hydrolysis allowed to proceed for about 24 hours. The solution was filtered, cooled and the reducing sugars estimated on a i-quotes as indicated before.

The values for the total, cold water-soluble and hot water-soluble carbohydrates and that for the fraction of sulphur in organic combination in the form of SO_4 and other forms for the different seaweeds during the 12 months of collection are given in Tables I to VIII. The seasonal variations in the ionic sulphur, total sulphate and total sulphur are represented in Figs. 1 to 11. The total agar content, the invert sugar corresponding to the hot water-soluble carbohydrate and the fraction of SO_4 that has gone into organic combination are plotted in Figs. 12 to 17.

Discussion of Results

Ionic sulphate in the algæ represents the first stage of absorption of the element sulphur from water. It may be seen form Figs. 1 to 11 that the quantity of ionic sulphate varies within wide range depending on the type of the alga and the time of collection, and sometimes it constitutes more

TABLE I

Sulphur and sugar contents in seaweeds collected during April, 1952

Name of Species	Sugar Content			Hot water-soluble	Sulphur	
	Total Sugar	Cold water-soluble			SO_4 in organic combination	S in other forms of combination
		Simple	Total			
C. linum ..	16·16	1·10	2·00	6·86	1·11	1·09
G. lichenoides ..	18·96	0·80	2·48	13·66	2·91	0·76
C. dasyphylla ..	14·00	0·18	2·16	10·98	1·86	1·62
E. intestinalis ..	14·70	Nil	1·20	6·36	2·01	1·95
A. spicifera ..	14·30	1·00	3·45	7·15	1·55	0·58
L. papillosa ..	12·12	0·54	2·70	5·00	1·61	1·85
H. musciformis ..	12·00	Nil	2·40	8·00	0·96	1·26
S. filiforme ..	16·89	0·90	2·60	9·80	1·95	1·32
S. furcellatum ..	11·40	0·75	2·00	8·90	1·30	0·75
R. intricata ..	13·70	0·88	3·60	3·80	0·35	0·07
P. australis ..	10·75	1·50	2·40	2·45	0·32	0·93

TABLE II

Sulphur and sugar contents in seaweeds collected during May, 1952

Name of Species	Sugar Content			Hot water-soluble	Sulphur	
	Total Sugar	Cold water-soluble			SO_4 in organic combination	S in other forms of combination
		Simple	Total			
C. linum ..	15·00	1·00	1·21	6·49	1·89	0·36
G. lichenoides ..	17·80	Nil	2·32	11·60	3·15	0·92
C. dasyphylla ..	12·94	0·50	1·61	9·20	2·02	0·14
E. intestinalis ..	21·40	1·00	1·87	7·15	1·88	0·87
A. spicifera ..	14·66	0·54	2·86	6·70	1·65	1·15
L. papillosa ..	12·94	Nil	3·59	4·96	1·89	1·81
H. musciformis ..	8·72	Nil	1·25	8·25	1·65	1·08
S. filiforme ..	13·74	0·75	1·65	8·40	..	..
S. furcellatum ..	16·48	Nil	1·34	8·18	1·60	1·00
R. intricata ..	15·20	Nil	4·69	5·20	0·05	0·82
P. australis ..	5·43	Nil	2·77	3·32	0·01	0·65

TABLE III

Sulphur and sugar contents in seaweeds collected during June, 1952

Name of Species	Sugar Content			Hot water-soluble	Sulphur	
	Total Sugar	Cold water-soluble			SO_4 in organic combination	S in other forms of combination
		Simple	Total			
C. linum ..	14·55	0·40	1·07	5·89	0·91	0·05
G. lichenoides ..	..	Nil	..	..	2·72	1·31
C. dasyphylla ..	11·15	0·50	1·52	5·89	1·49	0·92
E. intestinalis ..	10·08	0·75	0·63	5·60	0·67	0·49
A. spicifera ..	19·70	0·61	1·95	8·25	1·23	1·51
L. papillosa ..	8·63	Nil	2·23	4·50	1·46	1·67
H. musciformis ..	9·40	Nil	1·83	6·80	1·41	1·92
S. filiforme ..	11·45	0·44	1·49	7·20	1·20	1·57
S. furcellatum ..	18·90	Nil	1·96	9·88	0·47	0·69
R. intricata ..	16·54	Nil	3·50	3·68	0·51	0·07
P. australis ..	9·67	Nil	3·13	4·32	Nil	1·27

TABLE IV

Sulphur and sugar contents in seaweeds collected during July, 1952

Name of Species	Sugar Content			Hot water-soluble	Sulphur	
	Total Sugar	Cold water-soluble			SO_4 in organic combination	S in other forms of combination
		Simple	Total			
C. linum ..	16·17	0·43	1·00	6·30	0·42	0·51
G. lichenoides ..	15·17	Nil	0·89	9·20	2·15	1·07
C. dasyphylla ..	13·24	0·60	0·70	9·01	1·60	0·88
E. intestinalis ..	14·76	0·42	1·23	8·00	0·84	0·40
A. spicifera ..	13·14	Nil	0·89	8·01	1·29	1·16
L. papillosa ..	13·20	Nil	1·20	7·80	1·38	1·67
H. musciformis ..	12·34	0·62	0·80	6·70	1·96	1·57
S. filiforme ..	15·00	0·60	0·76	9·40	0·99	0·89
S. furcellatum ..	17·00	Nil	2·41	10·08	1·63	0·95
R. intricata ..	7·00	Nil	1·20	1·46	0·29	0·71
P. australis ..	9·20	Nil	2·30	2·68	0·36	1·15

TABLE V

Sulphur and sugar contents in seaweeds collected during August, 1952

Name of Species	Sugar Content			Hot water-soluble	Sulphur	
	Total Sugar	Cold water-soluble			SO_4 in organic combination	S in other forms of combination
		Simple	Total			
C. linum ..	12·37	1·08	1·80	6·40	0·25	1·62
G. lichenoides ..	14·00	0·80	1·64	8·20	2·00	1·35
C. dasyphylla ..	8·25	0·50	1·34	3·59	2·71	1·14
E. intestinalis ..	12·08	1·18	2·61	8·00	0·51	1·21
A. spicifera ..	13·14	0·80	1·00	7·30	1·49	0·78
L. papillosa ..	10·37	1·08	2·23	6·17	1·44	1·46
H. musciformis ..	12·00	1·20	2·68	8·25	1·59	2·14
S. filiforme ..	13·00	0·70	1·20	7·00	1·46	0·63
S. furcellatum ..	17·88	1·47	3·13	8·25	1·39	0·24
R. intricata ..	11·30	1·68	2·80	3·10	0·11	0·62
P. australis ..	12·70	Nil	3·60	3·65	0·66	0·82

TABLE VI

Sulphur and sugar contents in seaweeds collected during September, 1952

Name of Species	Sugar Content			Hot water-soluble	Sulphur	
	Total Sugar	Cold water-soluble			SO_4 in organic combination	S in other forms of combination
		Simple	Total			
C. linum ..	13·56	1·00	2·00	6·00	0·06	0·28
G. lichenoides ..	14·10	1·20	2·50	8·70	1·54	0·89
C. dasyphylla ..	8·34	1·60	4·41	6·80	1·01	1·63
E. intestinalis ..	12·48	0·98	2·70	8·30	0·41	0·22
A. spicifera ..	12·00	0·90	2·30	6·80	1·18	0·88
L. papillosa ..	12·54	0·64	0·89	4·80	0·24	2·84
H. musciformis ..	14·76	0·43	0·89	5·40	1·82	0·31
S. filiforme ..	12·36	1·00	1·23	5·40	0·85	0·79
S. furcellatum ..	7·30	1·12	2·68	6·93	0·95	0·67
R. intricata ..	13·14	1·64	3·59	4·40	0·16	0·44
P. australis ..	15·17	0·80	3·89	4·20	0·70	0·69

TABLE VII

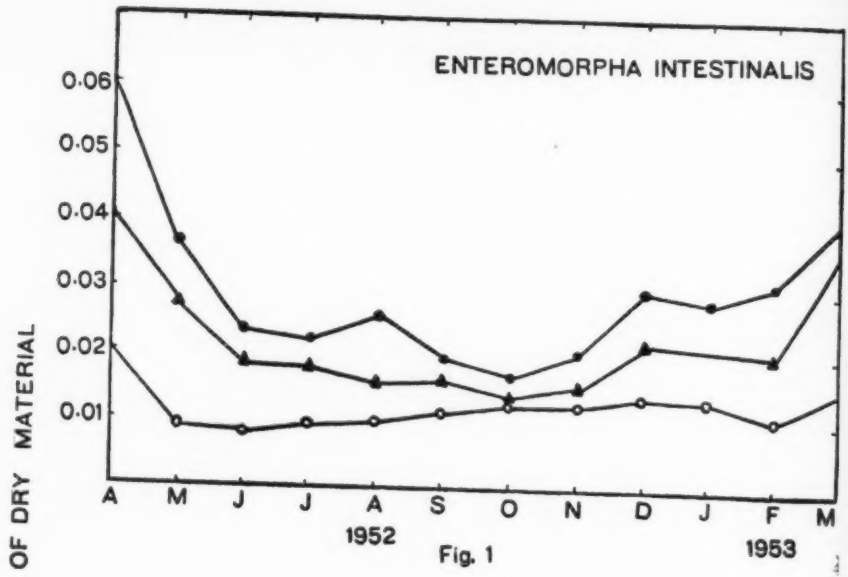
Sulphur and sugar contents in seaweeds collected during December, 1952

Name of Species	Sugar Content			Hot water soluble	Sulphur	
	Total Sugar	Cold water-soluble			SO_4 in organic combination	S in other forms of combination
		Simple	Total			
C. linum ..	13·06	..	1·89	5·83	0·32	0·23
G. lichenoides ..	13·10	1·23	3·20	6·40	0·98	1·16
E. dasyphylla ..	7·30	1·05	4·50	7·20	1·19	2·13
C. intestinalis ..	15·17	1·03	4·04	6·80	0·83	0·80
A. spicifera ..	17·00	0·83	1·34	5·56	1·36	0·36
L. papillosa ..	14·23	0·94	1·79	5·00	0·96	0·94
H. musciformis ..	5·43	Nil	Nil	2·40	0·32	1·36
S. filiforme ..	12·00	1·86	3·00	6·20	0·52	0·78
S. furcellatum ..	12·14	2·00	3·04	7·80	0·40	0·18
R. intricata ..	16·18	0·93	3·80	3·90	0·09	0·29
P. australis ..	16·00	1·00	3·70	4·20	0·20	0·98

TABLE VIII

Sulphur and sugar contents in seaweeds collected during March, 1953

Name of Species	Sugar Content			Hot water-soluble	Sulphur	
	Total Sugar	Cold water-soluble			SO_4 in organic combination	S in other forms of combination
		Simple	Total			
C. linum ..	14·16	1·09	2·20	7·09	1·18	1·06
G. lichenoides ..	22·48	0·73	2·40	15·35	0·94	1·44
C. dasyphylla ..	17·64	1·00	2·70	13·70	1·58	2·12
E. intestinalis ..	20·60	Nil	0·41	5·06	2·05	0·50
A. spicifera ..	11·86	0·50	1·06	6·08	1·20	0·66
L. papillosa ..	11·74	0·81	3·32	4·96	1·10	1·34
H. musciformis ..	10·76	0·80	1·05	8·82	1·13	0·37
S. filiforme ..	25·90	1·00	2·32	14·55	1·37	0·47
S. furcellatum ..	19·75	0·50	2·41	12·50	1·28	1·40
R. intricata ..	11·74	Nil	2·59	2·60	0·32	0·68
P. australis ..	11·15	Nil	1·58	1·64	0·07	0·28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0·01
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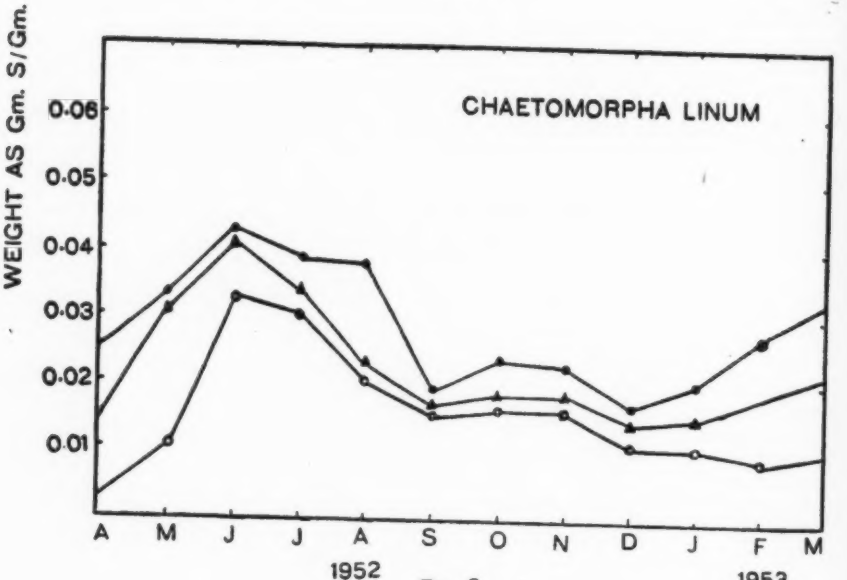
1952
1953

Fig. 1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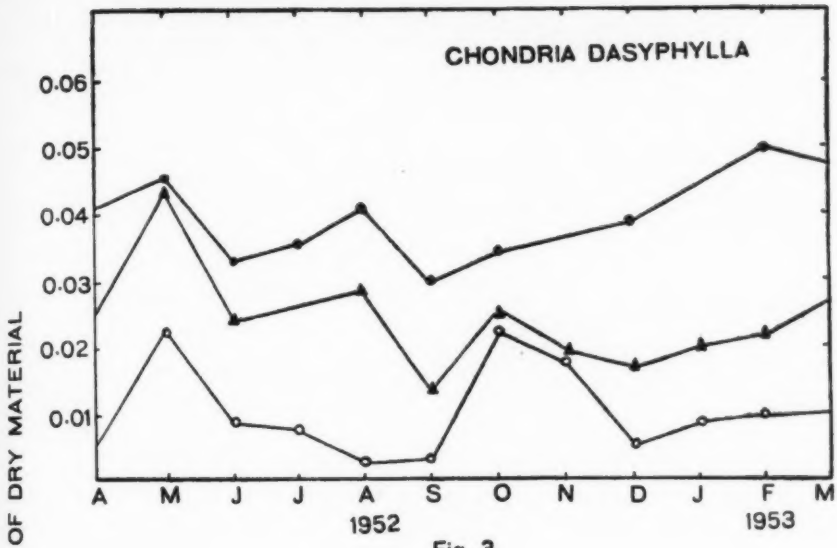
1952
1953

Fig. 2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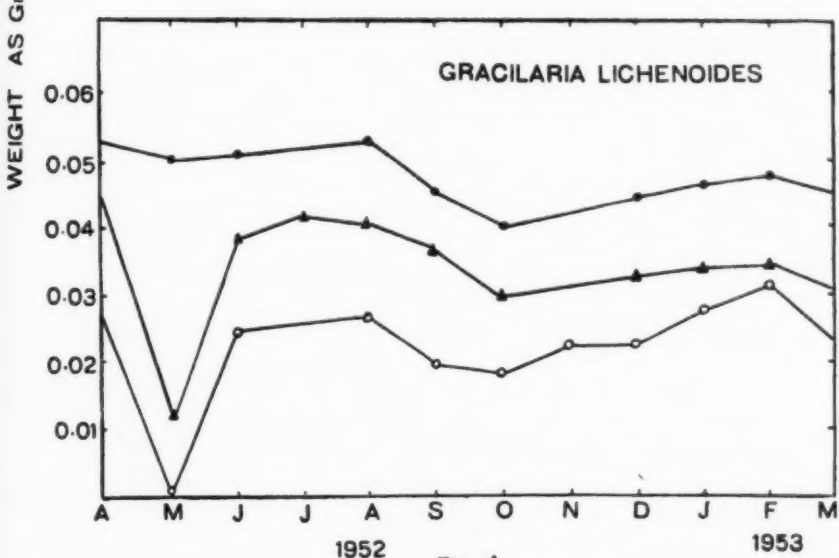
A M J J A S O N D J F M
1952
1953

Fig. 3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0·01
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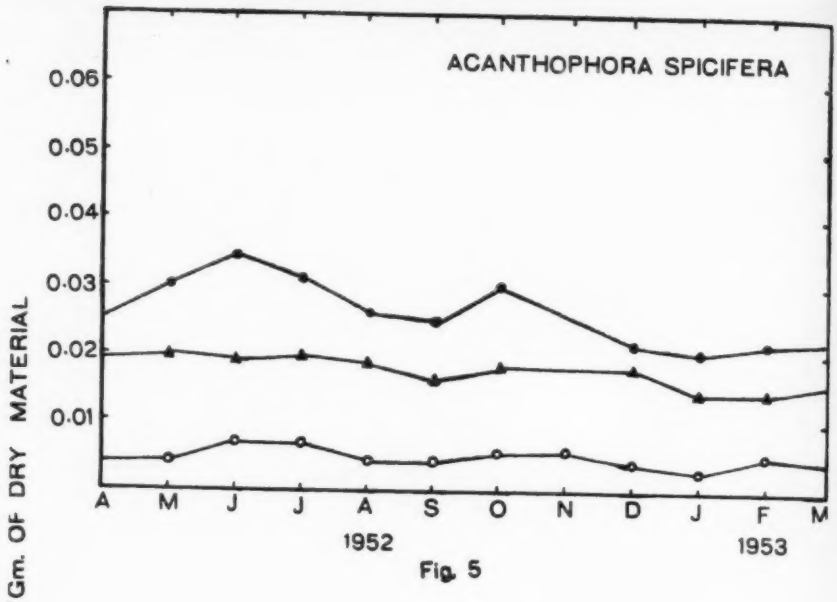
1952
1953

Fig. 4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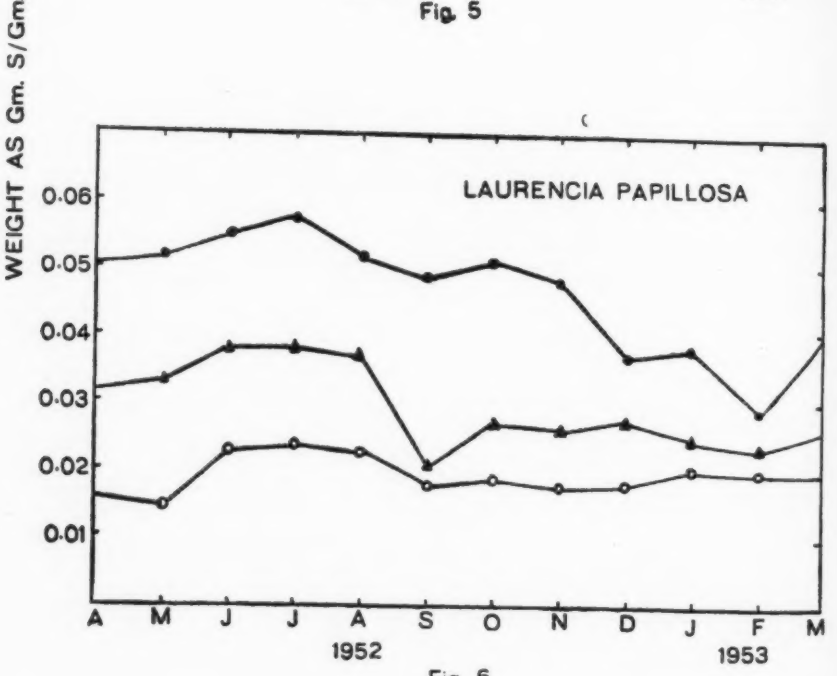
1952
1953

Fig. 5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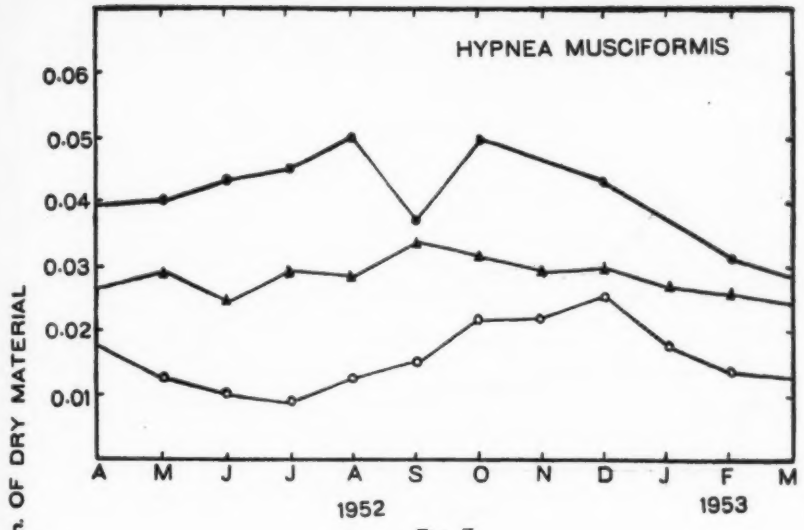
A M J J A S O N D J F M
1952
1953

Fig. 6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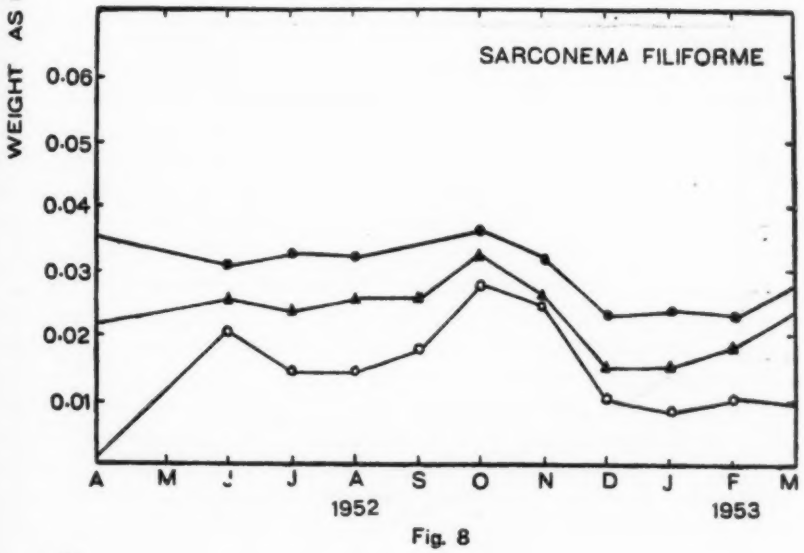
0·01
A M J J A S O N D J F M
1952
1953

Fig. 7


SARCONEMA FILIFORME
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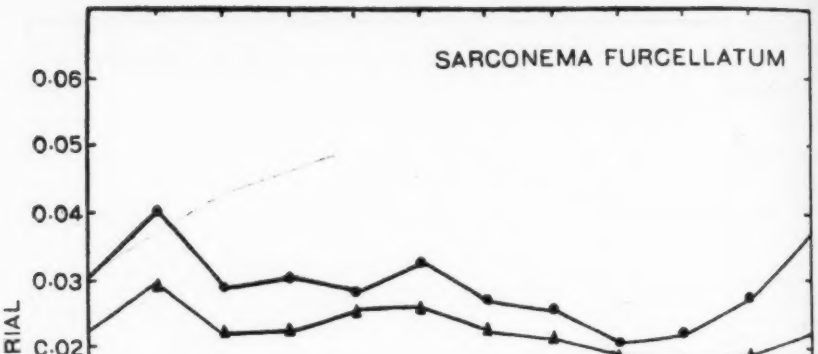
0·02
0·01
A M J J A S O N D J F M
1952
1953

Fig. 8


SARCONEMA FURCELLATUM
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0·05
0·04
0·03
0·02
0·01
A M J J A S O N D J F M
1952
1953

Fig. 9

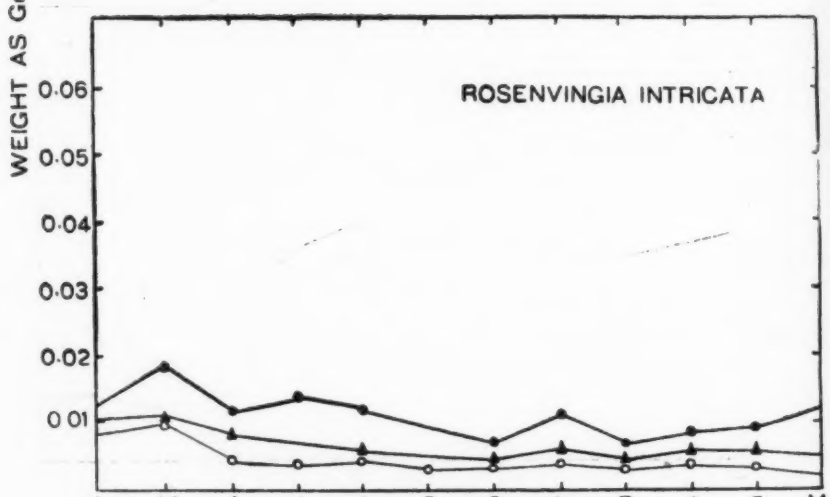
WEIGHT AS Gm. S/Gm. OF DRY MATERIAL
ROSENVINGIA INTRICATA
0·06
0·05
0·04
0·03
0·02
0 01
A M J J A S O N D J F M
1952
1953

Fig. 10

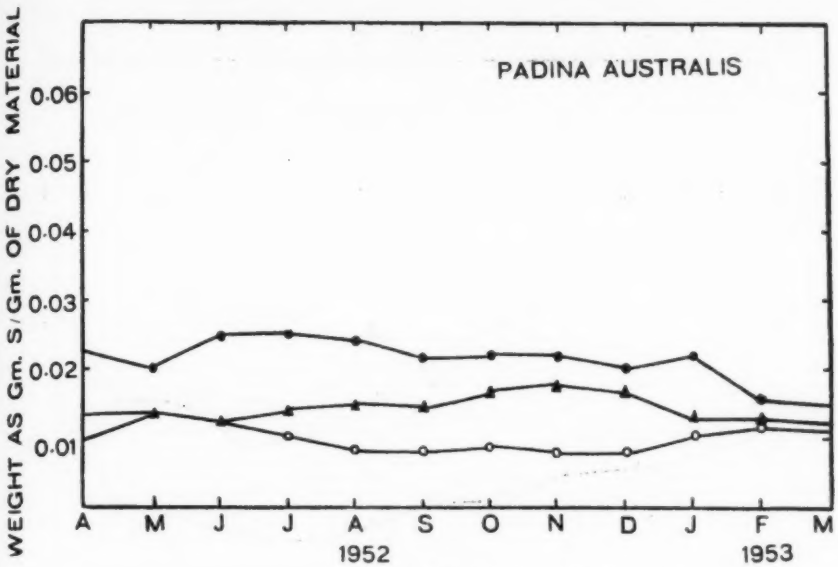

Fig. 11

than 70% of the total sulphur content of the algæ. For example in *C. linum* collected in June ionic sulphate constitutes 76·3% of the total sulphur, while in *G. lichenoides* collected in April and *A. spicifera* collected in May the corresponding figures are 50·7% and 52·2% respectively. Further in the collection made after September when the algæ are in the young stage the major portion of the sulphur is made up of the free ionic sulphate. Incidentally this increase in the absorption of SO_4 may be responsible for the less absorption of Cl, accumulation of which is considered injurious to the algæ. The difference between the ionic and the total sulphate gives the fraction that is in combination with the carbohydrate of the algæ. Similarly the difference between the total sulphate sulphur and the total sulphur gives the amount of sulphur that has gone into other forms of combinations—probably in the protein fraction of the algæ as sulphur-containing amino acids. The variations in these fractions of sulphur are quite distinct and can be correlated with the different growth stages of the plants and their organic constituents. Thus for example in *C. dasyphylla* there is a definite increase in the "protein sulphur" content corresponding to an increase in the total nitrogen content (Krishna Pillai, 1957) reaching a maximum in February, thereafter decreasing suddenly until the difference becomes least in May when the nitrogen content also falls to a minimum. The SO_4

Fig. 12

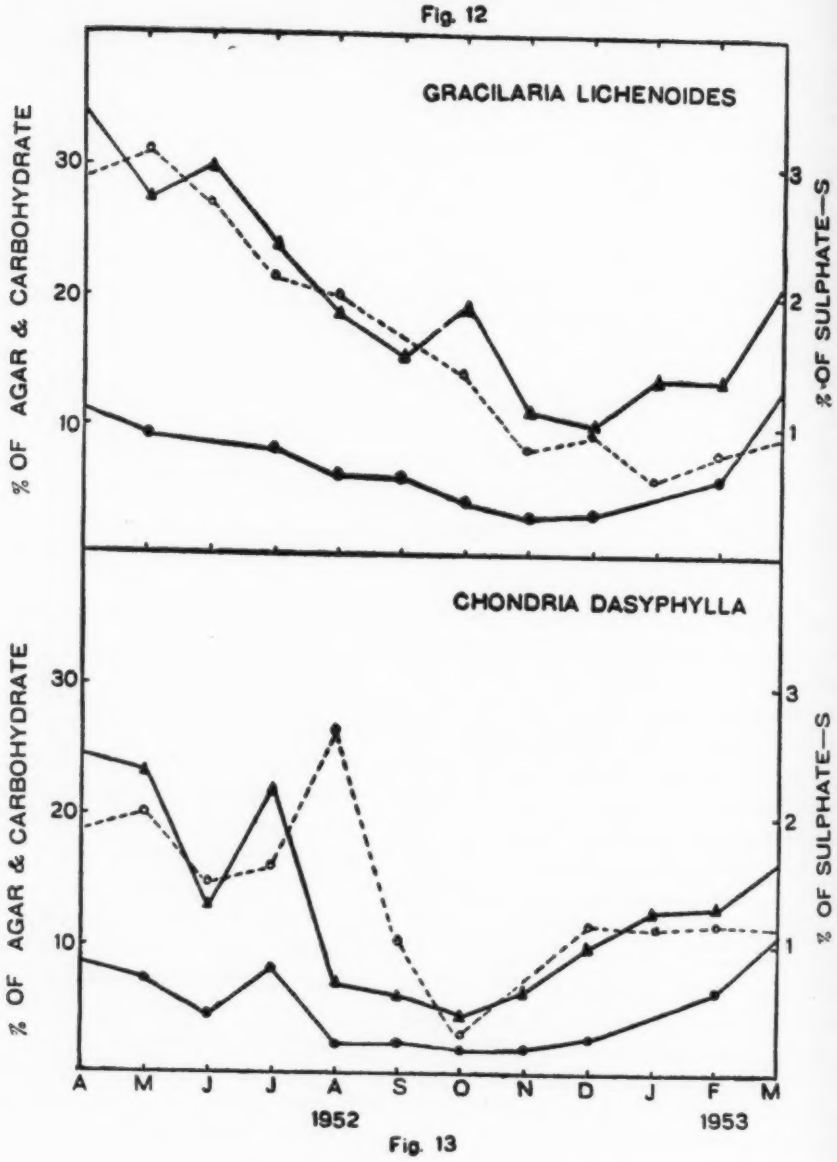
GRACILARIA LICHENOIDES
CHONDRIA DASYPHYLLA
% OF AGAR & CARBOHYDRATE
% OF SULPHATE—S
30
20
10
3
2
1
A M J J A S O N D J F M
1952
1953

Fig. 13

Fig. 14

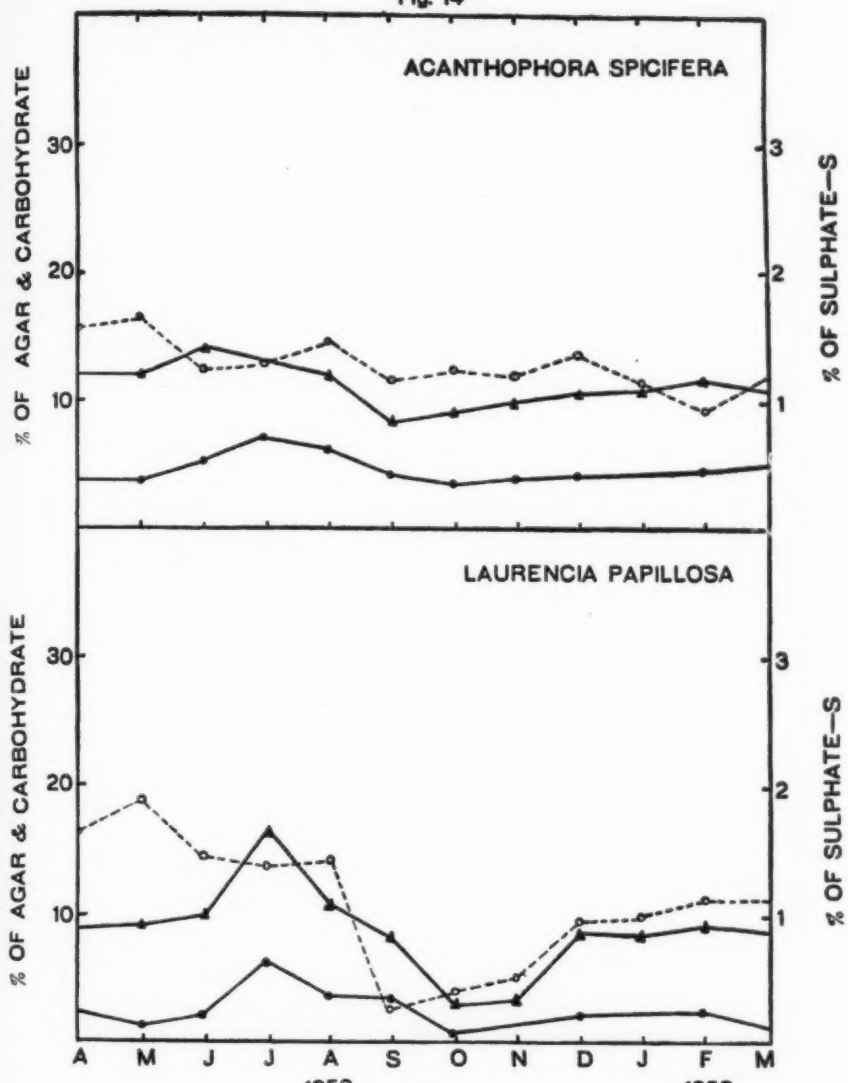
ACANTHOPHORA SPICIFERA
% OF AGAR & CARBOHYDRATE
% OF SULPHATE—S
30
20
10
3
2
1
LAURENCIA PAPILLOSA
% OF AGAR & CARBOHYDRATE
% OF SULPHATE—S
30
20
10
3
2
1
A M J J A S O N D J F M
1952
1953

Fig. 15

Fig. 16

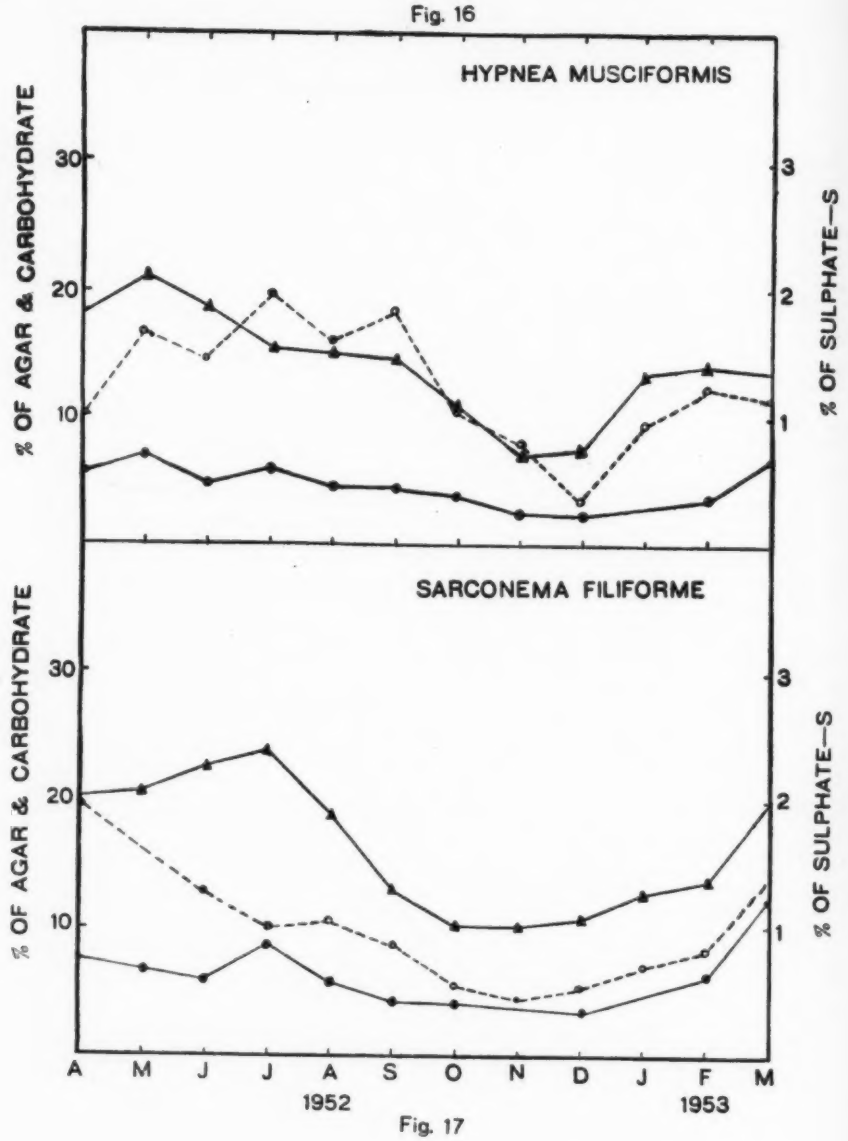
HYPNEA MUSCIFORMIS
% OF AGAR & CARBOHYDRATE
% OF SULPHATE—S
30
20
10
3
2
1
SARCONEMA FILIFORME
% OF AGAR & CARBOHYDRATE
% OF SULPHATE—S
30
20
10
3
2
1
A M J J A S O N D J F M
1952
1953

Fig. 17

fraction in organic combination along with the carbohydrate also follows a somewhat parallel course but in the reverse order. In October–November the amount of SO_4 in organic combination is a minimum but it increases from that period until it gives the maximum values during the months of May–August. This period of maximum corresponds to the time when the alga becomes mature and when it contains the minimum amount of nitrogen. It may be seen from Figs. 12 to 17 that the agar content of the red seaweeds also follows the same cycle of changes giving minimum values in October–November and maximum in March–July. In the case of *G. lichenoides* the variations in the different fractions are almost similar to that found in *C. dasyphylla* except that the protein fraction is at a maximum in May even though May–August is the period when the total nitrogen content is a minimum. It is interesting to note that the more the absorption of the SO_4 into the carbohydrate molecule the less is the total nitrogen content in the red seaweeds especially in the agarophytes. It may also be noted that SO_4 fraction in organic combination is at a minimum in *G. lichenoides* during the months of December–February, thereby showing that the agar content during this initial stages of growth of the alga will be low. This is found to be true when the values for the agar content of the species for the above period is examined (Fig. 12). In the case of *A. spicifera*, and *L. papillosa* there is not much of a seasonal cycle in the SO_4 content. The SO_4 in organic combination does not show any significant variations (Fig. 5) and likewise the agar content also stands very low (variation between 8·6 and 14·3% in *A. spicifera* and 3·0 and 16·8% in *L. papillosa*) though maximum values are obtained during may–July. In the case of these two algæ most of the sulphur remains either in the protein fraction or in the water-soluble form itself throughout the period. In *H. musciformis*, *S. filiforme* and *S. furcellatum* also the minimum values for the SO_4 in organic combination is met with in December, when the plants are young while this fraction increases slowly afterwards (corresponding to a decrease in the ionic SO_4) giving maximum values during April–August. During December most of the SO_4 will invariably be in the free, water-soluble form; but the proportion goes down afterwards.

In the two species of Chlorophyceæ studied most of the sulphur is present in the ionic form throughout the different stages of growth. The differences between the total sulphate and the ionic sulphate are comparatively very little. This indicates that although sulphate is accumulated in the green species it does not enter into organic combination as sulphate. But normal accumulation of sulphate may be necessary for the ionic equilibrium in the cells as well as for the synthesis of sulphur-containing amino acids.

The brown species *R. intricata* and *P. australis* stand separate in their sulphur metabolism, as may be seen from the tables and the Figs. (10 and 11). The total sulphur content in these two species is very low compared to the green and the red species mentioned above. Further there is practically no difference between the ionic sulphate and the total sulphate thereby indicating that sulphur does not go into organic combination in the form of SO_4 in these algæ. But a portion of the small quantity of the sulphur seems to undergo other forms of organic combination, probably as amino acids. It may also be seen from the figures that generally the total sulphur is greater in red and green seaweeds, the highest amount observed being 6·0% on dry weight basis in *E. intestinalis*. In *C. dasyphylla* the total sulphur varies between 3·2 and 5·0%, while the corresponding figures for the other species are as follows: *G. lichenoides*, 4·0 and 5·3%; *L. papillosa*, 2·8 and 5·7%; *A. spicifera*, 2·0 and 3·4%; *H. musciformis*, 2·8 and 5·0%; *S. filiforme*, 2·3 and 3·6%; *S. furcellatum*, 2·0 and 4·0%; *R. intricata*, 0·9 and 1·9% and *P. australis* 1·5 and 2·5%.

Tables I to VIII also give the values on the sugar content in the various fractions of the different seaweeds. The data show that the total sugar in all the species gives a maximum in April, May and June, the values decreasing afterwards until they give minimum values in December. In February the values again rise up. The maximum values have been recorded in the agarophytes (giving figures of 22·48% of invert sugar on the dry weight basis). In such of those red species as *A. spicifera* and *L. popillosa* which are known to be poor sources of agar the total reducing sugar in the algal hydrolysates is found to be very low.

The values for the simple sugars are obtained by inversion of the free sugars in the cold water-soluble portion of the algæ. Often very low values (maximum about 1% invert sugar) are obtained with maximum during the young stages of the plants. This indicates that in the young seaweeds simple sugars, the first products of photosynthesis, are present in larger quantities than in the older plants. As the plant grows more and more of the simple sugars are converted into higher polysaccharides.

From the Tables it is evident that a portion of the polysaccharides in the algæ comes out in cold water when the material is ground well with water. This fraction constitutes from 10 to 35% of the total sugars of the algæ. These are neither simple sugars, as they do not reduce Fehling's solution after simple inversion, nor complex molecules like agar, as agar is known to dissolve only in boiling water. However this fraction is a polysaccharide as it resolves into simple sugars on hydrolysis with acid. This fraction can

therefore be considered as the second stage of the conversion of simple sugars into complex carbohydrate molecule. Here again the values are found to be maximum during the early statges of the growth of the plants. The minimum values are recorded in May–July when the algæ are mature.

The reducing sugars corresponding to the total hot water-soluble fraction are given in the tables while the amount corresponding to the portion which will dissolve only in hot water is plotted in the graphs (Figs. 12 to 18). The values for the hot water-soluble portion change to a very great extent according to different species, the season and the growth stages of the plants. The maximum values are seen in March–June while the minimum values are found in August–December. The red species generally give the highest values for the aobve fraction constituting nearly 50 to 60% of the total sugar content of the algæ, while in the case of brown species the difference between the cold water and hot water-soluble portions is quite insignificant, thereby showing that the higher carbohydrates formed in the brown seaweeds are insoluble even in hot water. This observation is true because alginic acid which is the major constituent of brown seaweeds is insoluble in hot water.

Summary

Detailed investigations have been conducted on the partition of sulphur in different seaweeds common to the Indian coast. Among the seaweeds studied are two Chlorophyceæ, seven Rhodophyceæ and two Phæophyceæ. The amount of total sulphur, total sulphate and free sulphate in the different seaweeds were estimated periodically by analysing regular monthly collections. From this study it was also possible to follow the changes in the sulphur content of the algæ during the different stages of its growth and during different seasons. An attempt has also been made to bring out possible correlation between the different forms of sulphur and the carbohydrate content of the algæ.

It is observed that in the young plants most of the sulphur is present in the ionic form and that there is practically very little difference between the free sulphate and the total sulphate. As the algæ grow the difference between these two sulphate fractions increases steadily, except perhaps in the brown species, indicating that more and more sulphate passes from the free state into a state of combination with the organic compounds. It is also seen that the variation in the fraction of sulphate which is extracted only in hot water is directly proportional to the variations in the agar content of the seaweeds.

In the two species of Chlorophyceæ examined most of the sulphur exists in the free sulphate form throughout the year. Phæophyceæ contain only comparatively low amounts of sulphur and that practically no difference is noticed between the ionic sulphate and total sulphate.

The studies on the variations in the sugar content in the different fractions of the seaweed bring forth several interesting results. The total sugar content in the hydrolysate of the individual algæ shows a general maximum during the months April–June when the organic constituents are found to be maximum. Young specimens of almost all the seaweeds show varying amounts of simple reducing sugars in their cold water-soluble portions, indicating probably that the first products of photosynthesis in growing young seaweed are the simple sugars. As the algæ grow these simple sugars are converted to higher forms and in the mature algæ they are present only in very minute quantities.

ACKNOWLEDGEMENTS

I am grateful to Dr. N. K. Panikkar, Chief Research Officer, Central Marine Fisheries Research Station, Mandapam, for suggesting and directing the work. My thanks are also due to the Government of India (Ministry of Education) for the award of a Senior Scholarship during the course of the work.

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Explanation of Text-Figures

Figs. 1 to 11 ●——● Total Sulphur.

▲——▲ Total Sulphate—S.

○——○ Free ionic sulphate—S.

Figs. 12 to 17 ●——● Invert sugar corresponding to hot water-soluble carbohydrate

▲——▲ Agar.

○- - - -○ Sulphate-S corresponding to hot water-soluble portions.

SUPERPARASITISM IN *TRICHOGRAMMA EVANESCENS MINUTUM* RILEY (HYMENOPTERA: TRICHOGRAMMATIDAE), AN EGG PARASITE OF SUGARCANE AND MAIZE BORERS IN INDIA

I. Effect of Superparasitism

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Introduction

Within recent years a good deal of controversy has centred round the chalcid wasp *Trichogramma evanescens minutum* Riley. Holloway and Loftin (1919) state: "The chalcis-fly *Trichogramma minutum* is universally distributed in the sugarcane fields of Louisiana and of the Rio Grande Valley of Texas, and is a very important factor in the control of the moth borer (*Diatræa saccharalis* Fabr.)." Hinds and Spencer (1928) have shown with actual figures of *Trichogramma* colonisation experiments carried out in Louisiana that "the percentage of borer eggs parasitised in the colonised fields was continuously higher throughout the season than in the check field or the average of all uncolonised fields". The colonisation of *Trichogramma* in the cane fields in Barbados has proved to be a success and Tucker (1932) says: "So long as *Trichogramma* can be mass reared economically, distributed effectively, and timed to achieve maximum effective parasitism, there is sufficient evidence in Barbados to prove that it is securing control, but none whatever to prove that it should be condemned or discarded for any other control measures, not one of which has yet been proved effective."

Evans (1930) states that ovipositing many times in one and the same egg and causing superparasitism and ovipositing even in foreign bodies, such as waxy lumps (possibly excreta of the moth *Ephestia kuehniella* Zell.), etc., by *Trichogramma* cause a dissipation of its doubtful beneficial effect. Box (1932) observes that even a high degree of parasitism by *Trichogramma minutum* does not appreciably affect the sugarcane borer population in Antigua. Tucker (1932, 1933), in evaluating the usefulness of *Trichogramma*, has enumerated the objections raised against this parasite by other workers;

some of the objections are that it is limited by restricted range of operation, it is unable to find host eggs except by random movements and it is not specific to one host. Salt (1935) observes that female *Trichogramma* parasites are unable to distinguish between suitable and unsuitable non-parasitised hosts and will select the latter for oviposition if they are of larger size than the former. He further says that when false hosts, such as flour particles, sand particles, tiny glass globules, globules of mercury, etc., are given, *Trichogramma* attempts to oviposit even in these objects, where they are of larger dimensions than the true hosts, which are available along with them.

It may be seen from the review summarised above that there are two distinct schools of thought among the economic entomologists as to the exact role that this parasite can play in the biological control of pests, mainly borers. In view of these conflicting opinions and conclusions, the authors carried out a series of experiments on the varied habits of this parasite under laboratory conditions, which have not been undertaken in detail by previous workers with this particular objective.

It is well known that superparasitism is of common occurrence in *Trichogramma*, when mass multiplied in the laboratory. We have not got enough data to show whether the progeny that result from superparasitism have the same vitality and fecundity as those that singly emerge from a host egg. During the course of the present investigations some of these aspects, namely, the emergence, vigour, mating and fecundity of the progeny that result from superparasitism, have been studied in detail. The experiments and the conclusions arrived at are described in the following pages.

Material and Methods

Though in the Parasite Laboratory attached to the Division of Entomology, a large culture of the parasite *Trichogramma evanescens minutum* Riley and its laboratory host, *Corcyra cephalonica* (Staint.) were maintained, a separate culture of the parasites for these experiments was started with a single pair of a male and a female parasite in order to obtain a reasonably pure genetic stock.

Experiments

The following experiments were conducted to find out:

(*a*) the number of parasites that emerge from a single superparasitised egg of *Corcyra cephalonica* (Staint.),

(*b*) whether the progeny that result from superparasitism mate and successfully lay eggs, and

(*c*) the longevity and fecundity of individuals derived from superparasitised host eggs.

The experiments were conducted at a temperature of 25°–27° C. and at an average relative humidity of 75%. All the experiments were repeated at least six times.

OBSERVATIONS

Number of individuals that emerged from a single superparasitised host egg.—When the host egg was superparasitised, usually two *Trichogramma* emerged. These were either two males or two females, or a male and a female. Both had fully developed wings, or one had fully developed wings while the other had only crumpled reduced wings, or both had crumpled reduced wings. Sometimes only one parasite emerged from a host egg; but, on dissection, the egg was found to contain another partially developed individual. A few of the host eggs, from which only two *Trichogramma* individuals emerged, were found to contain, on dissection, a third partially developed form. Very rarely three parasites emerged from the same superparasitised host egg. In such cases, at least one of these had reduced wings only. In no case, however, more than three parasites ever emerged.

When the host eggs were subjected to prolonged exposure to parasites and after apparent heavy parasitisation, no emergence of the parasite took place. The host eggs, instead of turning black after parasite attack, became light brown and shrivelled. In other cases, even though the host eggs turned black, there was no emergence of the parasites; but, on dissection, the former were found to contain one or two partially developed forms.

Mating and egg laying of individuals that emerged from superparasitised host eggs.—Superparasitism has been found to have a definite influence on the selection of the opposite sex for mating. Mating between individuals derived from the same host egg was observed very rarely. A male parasite derived from a superparasitised host egg, however, often succeeded in mating with one derived from another superparasitised egg, but very rarely with a normally developed parasite,* because such a male is significantly smaller than normal males. Also, it was observed that if the male was normally developed and if the female was derived from a superparasitised egg and was very small compared to the normally developed females, mating between them did not successfully take place. But when the size of the

* Normally developed parasite refers to the one that emerges from a host egg without any superparasitism.

individuals derived from superparasitised eggs was big enough, they were able to mate with normally devleoped forms. Successful mating, however, was not obser ed between the largest and the smallest *Trichogramma*, although they attempted to mate.

When three individuals emerged from the same host egg, some did not show any tendency to mate, while others mated with individuals of about the same size.

Some of the females derived from superparasitised eggs laid eggs immediately after mating, while others did not lay any eggs even after mating.

Longevity of individuals derived from superparasitised host eggs.—The longevity varied just as in the case of normally developed parasites. It was usually less than twenty-four hours without food. As a rule, the males outlived the females.

Fecundity of parasites derived from superparasitised host eggs.—Mating between a male and a female derived from the same host egg, though very rare, was observed in two cases. One such female laid twenty-seven eggs while the other only nine eggs. When a female derived from a superparasitised host egg mated with a male derived from another superparasitised host egg, the maximum fecundity observed was thirty-nine and the minimum eight. The average of ten observations was twenty-two eggs.

Vitality of parasites that emerged from superparasitised host eggs.—The parasites that emerged from superparasitised eggs were not as active as normally developed parasites and took about twenty-four hours more to complete their development and emerge. Some of them were very inactive.

Progeny of individuals derived from superparasitised host eggs.—The progeny were apparently normal, although some females did not mate while others did not lay eggs even after mating.

Conclusions

From the above observations, it is seen that the number of adult *Trichogramma* that can emerge from a single superparasitised egg of *Corcyra cephalonica* (Staint.) varies from one to three, the usual number being two. In all cases of superparasitism, the progeny that successfully complete their development and emerge as adults, whether they are one, two or three, are always weak and usually have ill-developed wings, as compared with normally emerged individuals. Salt (1936) obtained upto two *Trichogramma evanescens* Westw. from a single egg of *Sitotroga cerealella* (Olivier).

When the host eggs were apparently heavily parasitised there was no emergence at all. Salt (1936) has observed this phenomenon in *Sitotroga cerealella* eggs also.

The competition between the developing parasites for the available amount of food in the host egg appears to be the factor that inhibits the normal development of the parasites, as Salt (1936) has stated. Ullyett (1936) has observed that the female of *Trichogramma lutea* Gir. has a conception of the size of the host and consequently it deposits only a single egg in the egg of *Sitotroga*, whereas it deposits three to four eggs in the larger egg of *Heliothis obsoleta* Fabr. However, in the case of laboratory breeding, on account of exposure to a large number of parasites and competition among them, superparasitism invariably results.

On account of their poor development, the mating habits are also adversely affected. Some do not show any tendency to mate; even if they mate, they very seldom do so with one emerged from the same host egg, always preferring one about their own size derived from another superparasitised host egg. They usually do not succeed in mating with a normally developed form of the opposite sex.

The fecundity is also very much affected. Such mated females lay only considerably fewer number of eggs than normal females.

THE PROBLEM OF ESTABLISHMENT OF THE PARASITE IN THE FIELD

Field colonisation of *Trichogramma evanescens minutum* Riley has been found unsuccessful in several cases, as has already been pointed out earlier in this paper. The observations made in the laboratory during the course of the present studies lead the authors to the conclusion that superparasitism is a potent factor which determines the success of colonisation.

In the liberations of the parasites in the field, the usual method employed is to expose the host eggs to the parasites under laboratory conditions and deploy these parasitised eggs on cards in the infested fields in a suitable manner. There is nothing to check or indicate whether the host eggs are superparasitised. If they are superparasitised, the observations made above would indicate a failure of the colonisation, on account of the following reasons:

1. The adults that emerge from the host eggs will be mostly defective ones; many do not have even a tendency to mate. Some will not lay eggs even after mating.

2. Even if the parasites by chance mate, the defective development, namely, ill-developed wings and inactive habit, stand in the way of their actively moving about in the field to find out the host eggs.

3. Even if the host eggs are sought out, the reduced fecundity will lead to a much lesser extent of parasitism.

From what has been stated above, it is more or less clear that if we want to colonise successfully infested fields with *Trichogramma* parasites and get the desired results, the parasites should be those that have emerged from such host eggs that have not been superparasitised at all, or even if superparasitised, it should be as low a percentage as possible. For this purpose special techniques, though very difficult, have to be evolved.

The population of the host eggs in the field at the time of liberation of the parasites is another important factor to be taken into consideration in successful colonisation. Should the population of the host eggs be too small in the field at the time of the first liberation, the parasites that emerge may superparasitise the available host eggs, which would result in encountering with the same problems as enumerated above. It is, therefore, essential to regulate the initial liberation of the parasites as well.

Summary

1. There are two diametrically opposite schools of thought as to the utility of *Trichogramma evanescens minutum* Riley for the biological control of insect pests.

2. Experiments have been conducted to assess the role of superparasitism in *Trichogramma* field colonisation.

3. The observations from the experiments show that as a result of superparasitism, though, one to three adult parasites may emerge from the egg of *Corcyra cephalonica* (Staint.), they are defective; they generally have ill-developed wings and are inactive. They mate with forms of about their own size only, if they show any tendency to mate at all; and even if they mate the fecundity is comparatively low.

4. The reason for the improper development of the parasites is attributed to the sharing of the limited amount of food in the host egg between the developing parasites.

5. These observations lead the authors to the conclusion that superparasitism is a very potent factor that determines the success or failure of the colonisation of *Trichogramma* in the field.

6. For successful colonisation, it is suggested that when the parasites are reared in the laboratory for mass liberation, every care should be taken to avoid superparasitism of the host eggs, for which special techniques have to be evolved; and also the initial population of the borer eggs in the field has to be taken into consideration at the time of the release.

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ON NEW LICHENS FROM THE HIMALAYAS—I

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(Communicated by Dr. S. N. Das-Gupta)

Introduction

During the last few years the author has made extensive collections of Lichens from various parts of India. These have been supplemented by many other collections presented to the author, noteworthy among which is the one by Prof. O. A. Höeg. Notes on some of these collections have already been published by Awasthi (1953, 1954) and Höeg (1953). This extensive collection of Indian Lichens in the author's herbarium, uptil now, comprises about 2,500 specimens, which have been serially numbered along with about 1,000 identified foreign lichens received on exchange or as gift.

The Indian collection is under careful investigation by the author. During the course of study, several species have been discovered which do not conform to any of the species known so far. After a thorough examination of these specimens and comparison with the related species they have been regarded as new species. These have also been confirmed by the Swedish Lichenologist, Dr. A. H. Magnusson, whose critical remarks (in correspondence) over the different species have been incorportaed in the paper.

The present paper and its series are intended to include only those species which have been found or will be found as new to Science, from the Himalayan region.

Method

The usual methods with which the Lichenologists are familiar have been adopted for the investigations. The anatomical details have been studied in free-hand sections in water as mounting medium. Treatment and reactions with the well-known chemical reagents used in Lichenology have also been tried, the abbreviations of which, used in the text, stand as follows:

KOH or K = aqueous solution of Potassium hydroxide; Cl = aqueous solution of Calcium hypochlorite; K (Cl) = K immediately followed by Cl; I = aqueous solution of Iodine (in a little Potassium iodide); Pd =

alcoholic solution of Paraphenylenediamine; + = positive reaction − = negative reaction and ± = more or less.

1. *Cetraria pallida* Awasthi sp. nov.

Thallus suberectus vel erectus, irregulariter divisus, glaucus vel flavescenti-albidus, laciniæ applanatæ, concavæ vel semicanaliculatæ, marginibus leviter undulatis, tenuiter fuscoatris, fibrillis atris rare instructæ, superficie lævigato pseudocyphellis atromarginatis ± dense ornatæ. Sterilis. Medulla Pd−.

Habitat.—On ground among mosses.

Locality.—E. Nepal; Topkegola, Thagalabhanjyang (in the main Himalayan range), altitude 13,500–14,000 ft.; coll. D. D. Awasthi; 29–5–1953: No. 2347 in Herb. author; Plate X, Fig. 1; TYPE.

Thallus suberect to erect, 2–3 (3·5) cm. tall, irregularly to subdichotomously laciniate, laciniæ irregular in outline, 2–6 mm. broad, applanate concave to semicanaliculate on the upper side, somewhat undulate marginally; margin irregular, brown black lined occasionally with brown black fibrils; fibrils 0·1–0·2 mm. long and 0·1 mm. thick. Thallus above lightly greenish pale to pale, smooth, ± faintly scrobiculate; below shining pale, occasionally brownish, with roundish brown black margined white specks (pseudocyphellæ), on an average about 0·5 mm. in diameter, sometimes the pseudocyphellæ confluent and appear as long streaks; the basal part of the laciniæ rarely reticulately ridged. No soredia and isidia.

Thallus 200–250 μ thick, plectenchymatously corticated, cortices of almost equal thickness (35–40 μ) on both the sides, cells somewhat indistinguishable on the exterior but 2–4 μ in size inwards; algal zone 30–40 μ thick with green (Protococcus) cells of average 10–12 μ in size; medulla white; medullary hyphæ 4–5 μ thick, moderately thick-walled (lumina 1·5–2 μ,) Upper cortex K + yellow; Cl−. Medulla K−, Cl−, K (Cl) + yellow. Pd−.

Thallus sterile.

"Belongs to subsection *Pseudocyphellatæ* Räs. and is related to *C. straminea* Vain., from the Philippines, but this species has broad ascendant lobes and grows on trees" (A. H. Magnusson).

2. *Cetraria nepalensis* Awasthi sp. nov.

Laciniæ thalli subsolitariæ, erectæ, elongatæ, subdichotome ramosæ, supra castaneæ, lævigatæ, nitidiusculæ, concavæ vel subplanæ, subtus pal-

lidiores, marginibus pycnidiis podicellatis, minutis instructæ, ad basin atro-fibrillosæ, pseudocyphellis deficientibus. Sterilis. Medulla K (Cl)—, Pd—.

Habitat.—On ground among mosses.

Locality.—E. Nepal, Topkegola, towards Thagalabhanjyang (in the main Himalayan range), altitude 15,000 ft.; coll.: D. D. Awasthi; 29–5–1953; No. 2373 in Herb. author; Plate X, Fig. 2; TYPE.

Thallus erect, 2–4 cm. tall, subdichotomously branched, laciniæ ± uniformly 2–3 (4) mm. broad, variously applanate concave to semicanaliculate on the upper side, margin somewhat undulate, minutely black dentate in the apical and black fibrillate in the basal parts; dents on an average 0·1 mm. in size and fibrils 0·25 × 0·1 mm. in size with pycnidia at the tips. Thallus above chestnutbrown; below usually lighter pale brown to concolorous as the upper side, both sides smooth shining; basal decaying parts of the laciniæ yellowish. No soredia, isidia and pseudocyphellæ.

Thallus thickest in the middle (160–200 μ) and gradually thinning outwards in the margin to 120–100 μ; plectenchymatous cortex of almost equal thickness (35–40 μ) on both the sides; cortex externally brownish, gradually lighter to colourless inwards, cells roundish, fairly large, 6–8 μ in size; algal zone 25–30 μ thick, algal cells green (Protococcus) upto 12 μ in size; medulla white, medullary hyphæ 4–5 μ thick, thick-walled and superficially finely granular. Upper cortex K—, Cl—, I—. Medulla K—, Cl—, K—, (Cl)—, Pd—.

Thallus sterile.

" Approaches certain specimens of *Cetraria delisei* var. *fastigiata* Del. in habitus but medulla in *nepalensis* K (Cl)— " (A. H. Magnusson).

3. *Physcia askotensis* Awasthi sp. nov.

Thallus suborbicularis, ambitu lobatus, glaucescenti-albus, lobis adpressis brevibus, subapplanatis vel subundulatis, inciso-crenatis, interdum leviter pruinosis, thallus subtus pallidus rhizinis pallidis instructus, cortice superiore KOH + flavo, medulla KOH immutata. Apothecia in centro crebra, majuscula, sessilia, disco fusco-atro vel atro, plano, margine prominenti flexuoso cincto. Hypothecium pallidum. Sporæ 8-næ ovali-ellipsoideæ, obscuratæ, uniseptatæ, pariete crasso.

Habitat.—On bark of tree trunk of *Alnus nepalensis.*

Locality.—N.W. Himalayas, Kumaon, Almora District, Askote, near village Sunkote, altitude 4,500 ft.; coll.: D. D. Awasthi; 2–5–1955; No. 3319

in Herb. author; Plate X, Fig. 3; TYPE; also from the same locality and habitat collected on 21–6–1954; No. 2687 in Herb. author; Para type.

Thallus foliose, suborbicular, Type *ca.* 10 cm. in size, but observed growing to larger dimensions than this, adpressed, laciniate lobate; lobes plane to irregularly undulated, 4–10 mm. broad, ascendent and somewhat imbricated along the margins, incised crenate, apex scarcely ascending. Thallus above glaucous white, pruinose, peripherally smooth and gradually minutely ridged reticulated to tubercled inwards (centrally); below light pale to greyish pale, rhizinose upto the margin; rhizinæ pale to greyish pale, branched, upto 1 mm. long and 0·1 mm. thick at the base, loosely adherent to the bark and thus thallus easily separable. No soredia and isidia.

Thallus 160–200 μ thick in the marginal lobes, paraplectenchymatously corticated on both the sides; upper cortex 25–35 μ thick, cells thin-walled, roundish to subangular 5–8 μ in size; algal zone 25–30 μ thick, algal cells green (Protococcus) upto 10 μ in size, roundish; medulla colourless of loosely interwoven hyphæ; hyphæ 4–4·5 μ thick, thick-walled (lumen 1 μ); lower cortex of almost the same thickness as the upper, paraplectenchymatous with somewhat thickened amorphous walls, cells 4–12 μ in size, irregular to roundish, larger cells towards medulla. Upper cortex K + yellow, Cl−, K (Cl) + yellow, I−. Medulla K−, Cl−, K (Cl)−, I−, Pd−.

Apothecia in the central part of the thallus, numerous, sessile, concave when young, plane to scarcely reflexed later, rounded to somewhat irregular in outline, usually mature ones upto 1·5 mm. in diameter; disc dark brown to black pruinose or not; margin prominent, flexuose and pruinose, rarely cracked. Thecium (hymenium) 90–120 μ thick, light brownish for about 10 μ in the upper part, I + blue, hypothecium pale, Asci clavate, 85–90 × 30–35 μ in size, 8-spored; spores biserially arranged, oval-ellipsoid, 2-celled, immature colourless, mature brown, thick-walled, ends rounded obtuse to acute, 24–32 × 10–13 μ in size, lumina of the spore cells varying, usually 6–7 μ in size.

" This species seems to be nearly akin to *Ph. biziana* (Mass.) Zahlbr. on account of the broad, pruinose laciniæ, the negative KOH-reaction in the medulla and the shape and arrangement of the apothecia, but is well distinguished in the smaller spores. The resemblance to the sterile *Ph. biziana* var. *aipolioides* Nadv. in his exsiccates No. 25 is rather great " (A. H. Magnusson).

4. *Physcia melanotricha* Awasthi sp. nov.

Thallus foliaceus, suborbicularis, obscure cinereofuscus vel fusco-ater, laciniatus, siccus, fragilis, humidis coriaceus, laciniæ sublineares, discretæ, profunde canaliculatæ, marginibus undulatis, superne lævigatæ, subtus atræ, dense atrofibrillosæ, reagentibus immutatæ. Cortex superior paraplectenchymatice cellulosus. Sterilis.

Habitat.—On water dipped wet sandy soil along with mosses and grasses.

Locality.—E. Nepal, near Topkegola, above the banks of Sajupokhari (*Saju* = proper name, *pokhari* = lake) in the main Himalayan range, altitude 14,500–15,000 ft.; coll.: D. D. Awasthi; 30–5–1953; No. 2410 in Herb. author; Plate X, Fig. 4; TYPE.

Thallus foliose suborbicular, 4–5 cm. in size, laciniate, subascending; laciniæ repeatedly subdichotomously branched, imbricated or superposed; almost uniformly 1·5–2 mm. (2·5–3·5 mm. before division) broad, apically narrower (upto 1 mm.); laciniæ canaliculate upto the apical end, even the smallest lobules concave on the upper side; margin smooth to minutely irregular. Thallus leathery coriaceous when wet and brittle in dry state; laciniæ in herbarium specimens sometimes twisted by about 180° exposing the under-side up. Thallus above smooth, dark greyish brown to brownish black, usually apical parts lighter in colour; below black, densely beset with black, branched hirsute and rough-surfaced rhizinæ of about 1 mm. length and 0·1 mm. thick at the base. No soredia and isidia.

Laciniæ 200–250 μ thick in the middle and thinner (100–80 μ) marginally; upper side paraplectenchymatously corticated; cortex 16–20 μ thick; cells roundish to subangular, thin-walled and 4–6 μ in size; algal zone 20–25 μ thick, algal cells green (Protococcus) upto 10 μ in size; medulla white of compact and conglutinated longitudinally disposed hyphæ (in transverse section the medulla often partly falsely appearing as paraplectenchymatous); medullary hyphæ 4 μ thick, thick-walled, (lumen 1 μ), smooth; under cortex $\pm$ plectenchymatous brown black and 5–8 μ thick. Upper cortex K−; Cl−, I−. Medulla K−, Cl−, K (Cl)−, Pd−.

Thallus sterile

" Belongs to *lithotea* group " (Magnusson).

The name—*melanotricha*—to this new species has been given on a suggestion from Dr. Magnusson.

5. *Physcia endococcina* (Korb.) Th. Fr. var. *latiloba* H. Magn. nov. var.

A Typo differt lobi thalli breves latique (2–5 mm.).

Habitat.—On bark of tree or on stones.

Locality.—N.W. Himalayas, Almora District, Askote, below village Sunkote, altitude 4,500 ft. (on bark of *Alnus nepalensis*); coll.: D. D. Awasthi; 2–6–1955; No. 3318 in Herb. author; Plate X, Fig. 5: TYPE; also from the same locality and habitat dated 21–6–1954 (No. 2699). From localities near about Askote—on bark of *Pyrus Pashia* (No. 3302), on *Sapium insigne* (No. 3303), and over stone (No. 3301).

Distribution.—" Found also in Hawaaiian Islands. The corticolous type seen from U. S. A., Maryland and Maine "—(A. H. Magnusson).

Thallus orbicular when small in even substratum and becoming suborbicular in larger dimensions, upto 10 cm. in diameter, adpressed, laciniate; laciniæ upto 5 mm. broad, crenate, dissected, ascending imbricate marginally. Thallus above ashy grey to greyish brown, smooth, no soredia and isidia; below black rhizinose; rhizinæ upto the margin, black, dense, and upto 2 mm. long.

Thallus about 200 μ thick, corticated on both the sides, upper cortex 40–45 μ thick, paraplectenchymatous, cells thin-walled, roundish to subangular, 6–8 μ in size; algal cells green, medulla yellowish in the upper about $\frac{1}{3}$–$\frac{1}{4}$ region and coccineus in the lower $\frac{2}{3}$–$\frac{3}{4}$ region, medullary hyphæ 3·5–4 μ thick, thin-walled; lower cortex 20–25 μ thick, dark brown to brown black, cells roundish to somewhat elongated, 8–12 μ in size. Upper cortex K + yellowish, Cl—. Medulla K + violet (due to the interaction the violet colour is also driven out of the medulla), Cl + dark violet to violet brown.

Apothecia numerous, crowded in the central part, superficial, sessile, beset with about 1 mm. long black cilia at the base, receptacle attaining a size of about 1·5 mm. in diameter, with prominent thalline margin; disc brown black to black, concave in young stages, plane later. Thecium (hymenium) about 100 μ thick, I + blue, epithecium yellowish; hypothecium yellowish, medulla coccineus. Asci club-shaped, 8 spored; spores brown 2-celled, oval ellipsoid, placodioid, thick-walled, 22–30 × 12–15 μ in size; paraphyses slender, simple, tip swollen.

6. *Anaptychia himalayensis* Awasthi sp. nov.

Thallus suborbicularis, laciniatus, cinereo-albus vel glauco-cinereus, laciniæ partim horizontales, ± imbricatæ, plerumque apicibus adscendentes,

partim suberectæ, lobis crenatis et varie tuberculosis, lævigatis, haud sorediosis, pycinidiis obscuris verruciformibus sæpe ornatis. Rhizinæ marginales obscuræ sæpe visibiles. Cortex KOH + flavus, I + cærulescens, hyphæ corticales longitudinaliter ± parallelæ. Apothecia numerosa, superficialia vel alte podicellata, majuscula, disco fusco pruinoso, immerso, margine alte prominente, laciniato. Spores 8-næ, fuscæ, diblastæ, placodiomorphæ, majusculæ.

Habitat.—Over stone in subshady place.

Locality.—N.W. Himalayas, Almora District, Askote, below village Naret, altitude 4,000–4,500 ft.; coll.: D. D. Awasthi; 28–5–1954; No. 2653 in Herb. author; Plate XI, Fig. 3: TYPE.

Thallus suborbicular, upto 4·5 cm. in size, adpressed, horizontal to apically partly subascending; laciniæ subdichotomously branched 1–2 mm. broad, convex on the upper side, imbricate to compactly interlocked with each other (chiefly in the central part) by the marginal cilia, apically subascending and bearing superficial to subterminal podicellate apothecia; often the horizontal part of thallus superposed by the dense, suberect lobes, secondary in nature of development, lobes small, 1–2 mm. broad, entire or crenate and divided; laciniæ and lobes with black pycnidial dots and often variously tubercled with pycnidial protuberances; laciniæ margin ciliate; cilia branched basally concolorous to the thallus and distally brown. Thallus above greyish white to glaucous grey, smooth waxy to scarcely pruinose in the marginal lobes; below white farinose, without rhizinæ. No soredia and isidia. Thallus usually has a brownish scum of *Scytonema* alga covering the marginal grooves in the central part.

Thallus corticated (pseudocortex) on the upper side and along the margins by longitudinally ± parallel hyphæ, thickness varies from 40–300 μ due to irregular reticulations in the inner outline of the cortex, the algal zone discontinuous, algal cells 12–15 μ in size, green (Protococcus), occur in groups in the grooves of the cortex, sometimes groups of algal cells as islands within the tissue of cortex; medulla thin of loosely interwoven superficially densely granular hyphæ. No lower cortex. Cortex K + yellow, Cl−, K (Cl) + yellow, I + violet blue to blue; medulla K−, Cl−, I−, Pd + orange yellow.

Apothecia numerous, superficial to subterminal on horizontal or suberect laciniæ, mature podicellate, suberect to erect peltate, upto 8 mm. in diameter; disc brown pruinose with an inner rim-like faintly orange-coloured margin, medullary in origin, and an outer proliferated thalline margin with 1–3 mm. long lobules, farinose on the inner and corticated

on the outer side, the lobules marginally ciliate, cilia of the same nature as in thallus. Apothecia arise as globular structures on the thallus with eciliate inflexed lobules in young stages; and later are plane to somewhat reflexed with ciliate lobules. Thecium (hymenium) 180–200 μ thick, brownish in the upper part and colourless inwards, I + blue, hypothecium yellowish in thin sections, brownish otherwise. Asci club-shaped, 120–140 × 20–25 μ in size, 8-spored; spores brown, oval ellipsoid, 2-celled, somewhat constricted at the septum, placodioid, thick-walled, 32–40 × 16–20 μ in size; paraphyses slender, simple, upto 2 μ thick.

This species is closely related to *A. podocarpa* (Bél.) Mass., but can easily be distinguished by its habitat, the habit of laciniæ, the I + blue or violet blue reaction of the cortex, the ciliate lobulate margin of apothecia and the smaller spores.

7. *Anaptychia pellucida* Awasthi sp. nov.

Thallus fruticulosus, rosulatus, cinereo-albus vel obscure cinereus, laciniæ subdichotome lobatæ, convexæ, basi subangustæ, superne latiores, fibrillis atris marginalibus vel superficialibus ornatæ, subtus albidæ, farinosæ vel reticulatim subnervosæ. Cortex superior KOH–, I + cærulescens, hyphæ longitudinaliter directæ. Apothecia breviter podicellata, margine elevato crenato haud fibrilloso, disco obscure fusco, leviter pruinoso vel nudo. Sporæ magnæ, 8-næ, obscuræ, placodiomorphæ, maculis pellucidis (cellulis indistinctis) instructæ.

Habitat.—On twigs of a shrub.

Locality.—E. Himalayas, Darjeeling, Tiger Hill, altitude 8,000 ft.; coll.: D. D. Awasthi; 10–10–1954; No. 3136 in Herb. author; Plate XI, Figs. 1 & 2: TYPE.

Thallus fruticulose, 4–6 cm. in size, rosulate, fixed to the substratum (twig) only centrally, subdichotomously branched, laciniate; laciniæ convex on the upper side by curving inwards of the margins, narrow basally and broad flattened upto 5 mm. upwards. Thallus above glaucous-ashy-white to dark grey or blackish, smooth to occasionally minutely brown black tubercled; tubercles pycnidial in nature, simple or confluent; thallus below white farinose and minutely reticulately nervose; fibrils along the margin and on the upper side, black, branched, thyrsoid, intricated and hirsute, upto 3 mm. long and 0·2–0·3 mm. thick at the base. No soredia and isidia.

Thallus corticated (pseduocortex) on the upper side and along the margins by longitudinally running thick-walled hyphæ, uppermost layer of

the cortex lightly brown, inner outline irregular and greatly reticulated, thus thickness varying between 40–275 μ, and often continuous upto the lower surface without any cover of medulla (these thick parts represent the superficially nervose reticulations); algal zone discontinuous, algal cells green (Protococcus) in irregular patches located in the concavities formed by the reticulated inner side of the cortex; algal cells 10–14 μ in size; medulla present and thin covering only the groups of algal cells; no lower cortex. Cortex K−, Cl−, K (Cl)−, I + violet blue to blue (upper surface yellowish); medulla K + scarcely yellowish Cl−, I−, Pd + yellow.

Apothecia superficial to subterminal, shortly podicellate, 2–3 mm. in diameter, disc dark brown, scarcely pruinose to nude; the margin thalline, entire in young stages and thin crenate later, without any fibrils; cortex of the receptacle 120–150 μ thick, I + deep blue. Thecium 260–300 μ thick, light brownish for about 40 μ in the upper part and colourless inwards, I + blue; hypothecium colourless in thin and yellowish in thick sections, I + light orange. Asci large, clavate to irregular (due to bulging spores) about 215 × 60 μ in size, 8-spored; spores large, ellipsoid, brown, 2-celled, placodioid with many globular pellucid areas towards the two ends (illusively globularly celled), 60–72 × 24–28 μ in size; paraphyses slender, simple, 2 μ thick. Pycnoconidia straight 3–4 × 1 μ and somewhat narrower in the middle region.

" Related to *A. podocarpa* but spores larger and in spots pellucid " (A. H. Magnusson). It can also be distinguished by the I + blue reaction in the cortex. It shows similarity to *A. comosa* in the presence of cilia on the upper surface but spores in *comosa* are much smaller.

The two preceding species of Anaptychia—*A. himalayensis* and *A. pellucida*—have shown an interesting reaction when treated with iodine solution. The cortical region in both of these develops a violet blue to blue colour by this treatment. The colouration is much pronounced in the cortical region of the apothecia. The only other Anaptychia with "a positive I-reaction, however, is recorded by Vainio in *A. obscurata* var. *serpens* " (Magnusson). Thus these two can easily be distinguished from the rest of the Anaptychia by this reaction:—Cortex I + blue.

8. *Peccania Höegii* Awasthi sp. nov.

Thallus effusus, subfruticulosus, olivæo-ater, partes thalli erectæ, breves, subcontiguæ vel approximatæ, teretes, superne crassiores, partes fertiles apice dilatatæ, apothecium unicum ferentes, disco concavo, cinereo-

suffuso, margine tumido atro cincto, algas cæruleo-virides minutas, glomeratas includente. Hypothecium pallidum, indistinctum. Paraphyses conglutinatæ, granulis obscuris nubilatæ, thecium inde indistincte visum. Asci rare evoluti, sporæ 8-næ, simplices, ellipsoideæ.

Habitat.—On hard chalky soil over stone (or boulders).

Locality.—N.W. Himalayas, Kangra District, Spiti Valley, Kaze, altitude 12,000 ft.; coll.: O. A. Höeg; 9–8–1952; No. 1653 in Herb. author; Plate XI, Figs. 4, 5 & 6: TYPE. Also from Kangra District, Pin River Valley, 1 mile south of Sangam, altitude 12,800 ft.; coll.: O. A. Höeg; 6–8–1952; No. 1629 in Herb. author.

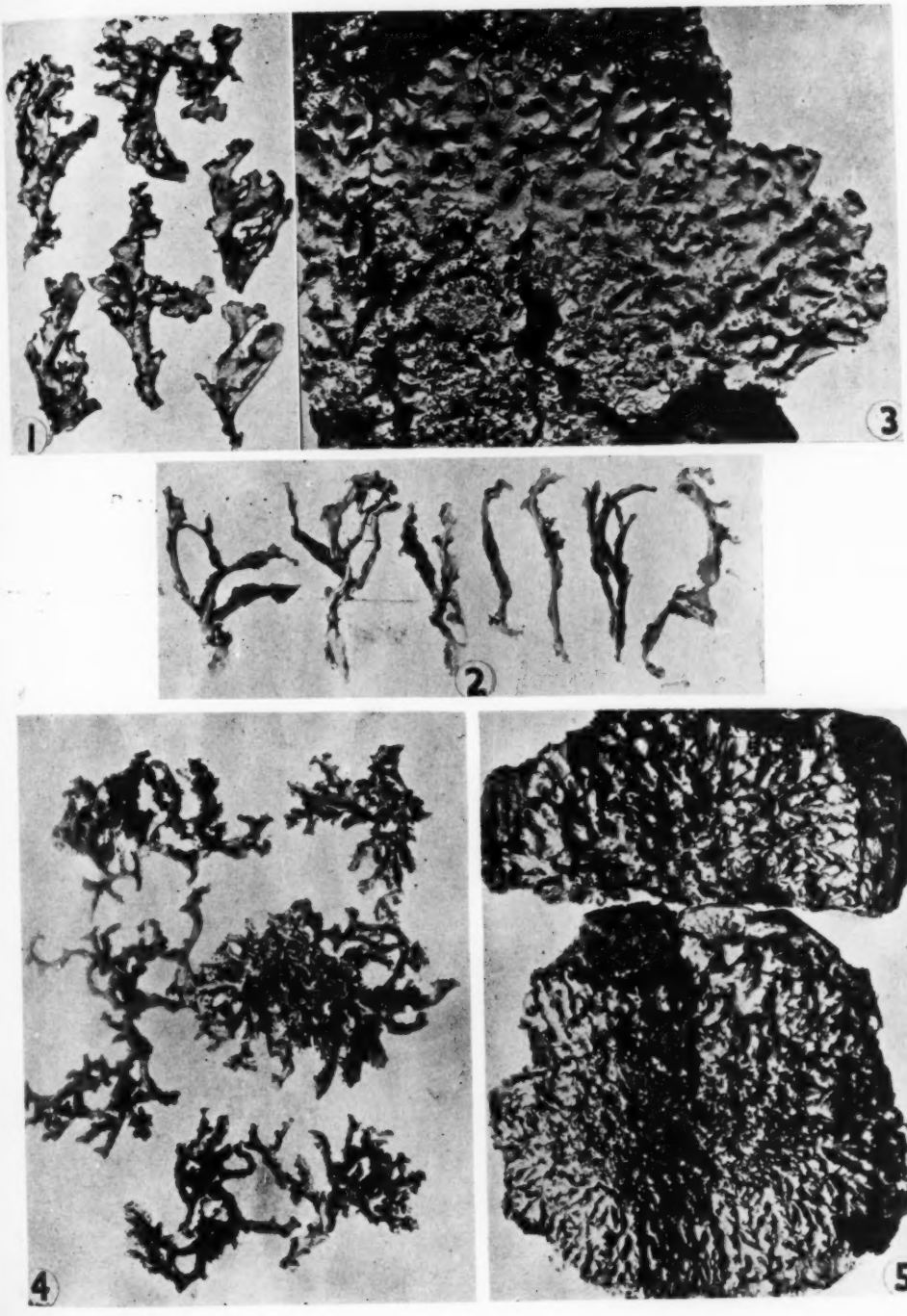
Thallus effuse, black, subfruticulose, consisting of the vertical thallus parts 0·6–1 mm. high, club-shaped, contiguous in groups or more or less isolated; at least top black, otherwise more or less suffused by greyish earthy particles. The hyphæ of the thallus densely traversing as deep as 2 mm. within the chalky soil, and are thick-walled, septate and 4–5 μ thick. Thallus in section yellowish brown and amorphous in the outermost 4–5 μ thick region, followed inwards by the gonidial zone of 80–100 μ thickness. Algal cells blue green 4–6 μ in size, and in well demarcated groups within a mucilaginous (?) type of substratum (matrix); medulla of dense hyphæ almost vertically disposed.

Apothecia apical on a 0·12 mm. thick erect stalk, about 0·8–1 mm. high; disc at first closed, open later, widening to about 0·6 mm. with concave mostly suffused rounded disc and turgid, black margin; KOH + yellow. Algal cells blue green present in the margin and in the stalk. Apothecial margin upto 160 μ broad, upper surface dark brown in water, laterally 100 μ thick with numerous blue green algæ 4–6 μ in diameter, in small dense groups. Thecium 80 (100) μ thick, I + blue, nubilated and dark from black brown spots between or on the paraphyses. Hypothecium paler brown, I–. Asci clavate, 45–60 × 12–15 μ, 8-spored, very rarely mature; spores not seen released outside the ascus, as observed within the ascus simple, colourless, oval ellipsoid, 10–12 × 6–8 μ in size. Paraphyses simple, 1 μ thick.

It is a minute lichen, interesting in having peculiar brown black nubilations in the thecium, a structure " never seen in any other lichen " (Magnusson). The specimen has been named after its collector, Prof. O. A. Höeg (Norway).

SUMMARY

The paper deals with 7 new species and one new variety of lichens from the Himalayan region. They are *Cetraria pallida* Awasthi, *C. nepalensis*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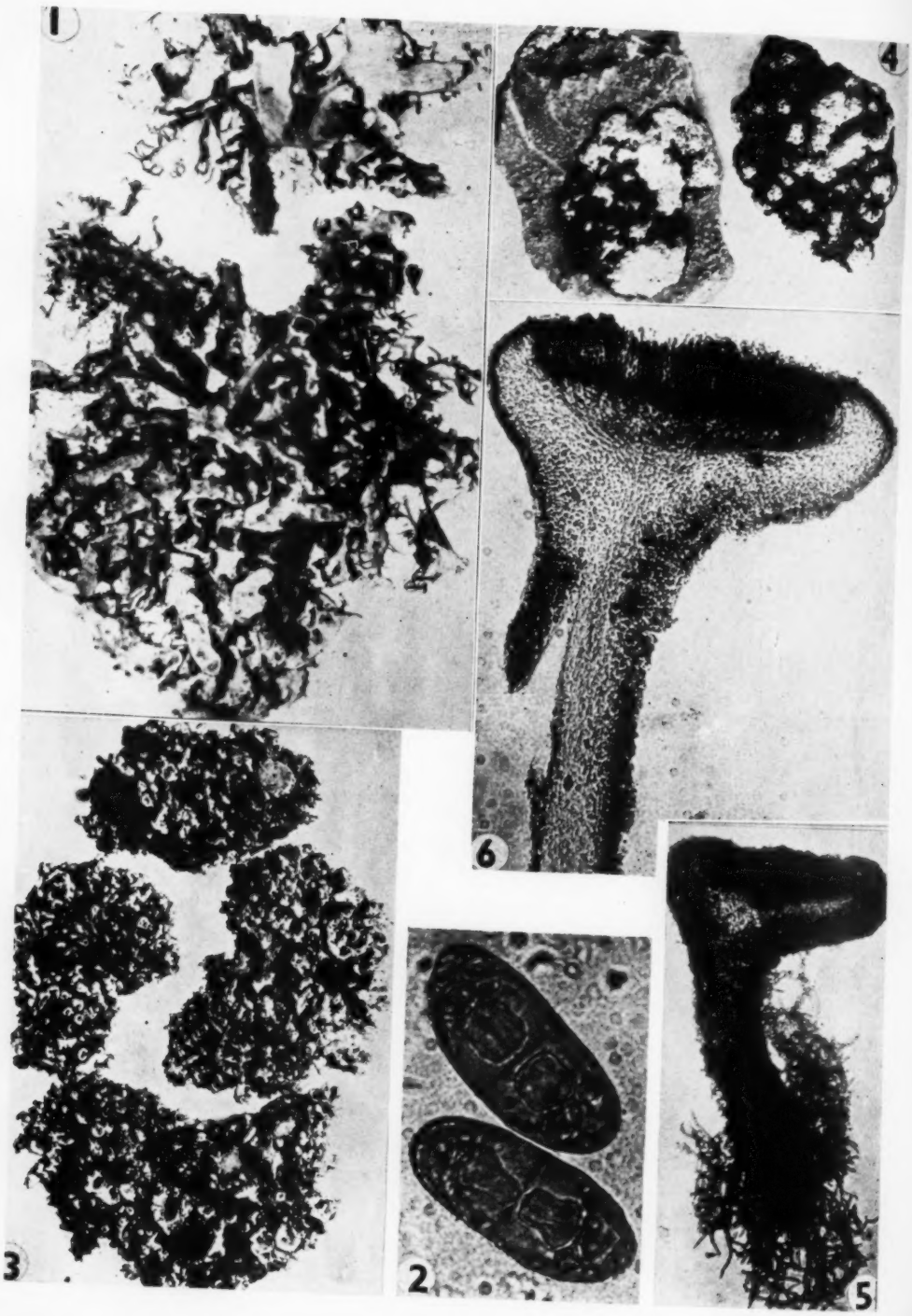
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Awasthi, *Physcia melanotricha* Awasthi, *Ph. askotensis* Awasthi, *Anaptychia himalayensis* Awasthi, *A. pellucida* Awasthi, *Peccania Höegii* Awasthi, and *Physcia endococcina* var. *latiloba* H. Magn. Mention may be made of the two interesting features, notably deep blue colouration in the cortical region of *Anaptychia himalayensis* and *A. pellucida* by the interaction of iodine solution and the strange dark nubilations in the thecium of *Peccania Höegii*.

Acknowledgments

The author takes this opportunity of expressing his grateful thanks to Dr. A. H. Magnusson (Sweden) for his keen interest, valuable suggestions, confirmation of the identifications and translating the diagnostic characters of the new species into Latin. The author is gratefully indebted to Prof. S. N. Das-Gupta, Professor of Botany, Lucknow University, for his constant encouragement and guidance in the prosecution of this work.

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Explanation of Plates

Plate X

Fig. 1. *Cetraria pallida* Awasthi (HOLOTYPE), *ca.*, ×1.

Fig. 2. *Cetraria nepalensis* Awasthi (HOLOTYPE), *ca.*, ×1.

Fig. 3. *Physcia askotensis* Awasthi (HOLOTYPE), *ca.*, ×1.

Fig. 4. *Physcia melanotricha* Awasthi (HOLOTYPE), *ca.*, ×1.

Fig. 5. *Physcia endococcina* var. *latiloba* H. Magn. (HOLOTYPE), *ca.*, ×1.

Plate XI

Fig. 1. *Anaptychia pellucida* Awasthi (HOLOTYPE), *ca.*, ×1½.

Fig. 2. *A. pellucida*—Spores (×600) showing pellucid areas within the spore cells.

Fig. 3. *Anaptychia himalayensis* Awasthi (HOLOTYPE), *ca.*, ×1.

Fig. 4. *Peccania Höegii* Awasthi (HOLOTYPE), *ca.*, ×1.

Fig. 5. *P. Höegii*—Vertical longitudinal section through the fructification (×65), showing the basal hyphæ.

Fig. 6. *P. Höegii*—Vertical longitudinal section through the apothecium (×125), showing algal zone in stalk and margin and the dark nubilated thecium.

STUDIES IN SAMPLING TECHNIQUE

VIII. Estimation of Incidence of Sugarcane Borers in a Field

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1. Introduction

Top, stem and root—the three types of borers are the most damaging pests of sugarcane crop in this part of the country. The intensity of activity of these pests may be measured by percentage length of cane damaged or percentage nodes infested. Khanna and Banerjee[1] have worked out sampling technique for estimation of *length-basis* incidence in a field combined for all the three types of borers, taking incidence per clump as the ultimate variate. In this paper a smapling technique with sample sizes has been evolved for estimation of incidence of each type of borers, the ultimate variate being *node-basis* incidence per cane.

2. Materials and Methods

The data of this investigation were collected at Pusa by the staff of Entomology Division of the station during the harvest season of 1951–52 crop. Four plots of sugarcane (each of 72′ × 18′ in size) marked out of fields, two under variety Co. 453 and two under B.O. 11, were completely enumerated in respect of total number of nodes of each cane and number of nodes affected by each type of borers, if any. The percentage incidence figures were calculated after Bartlett's correction and subsequently transformed on angular scale for statistical analysis.[2] Each of the plots had six rows 3′ apart and so the total row length was 432′ which was divided into units of 3′, 6′ and 9′. Each type of division has thrown the finite population of canes into a number of unequal clusters (clusters that contain unequal number of canes). As a measure of homogeneity the statistic δ as defined below has been calculated in respect of each type of clusters:

$$\delta = \frac{\sum_i M_i (\bar{Y}_i - \bar{Y})^2 - \sum_i \sum_j (y_{ij} - \bar{Y})^2}{\sum_i (M_i - 1) \sum_j (y_{ij} - \bar{Y})^2}$$

where,

M_i = number of canes in the i-th cluster.

y_{ij} = j-th cane in the i-th cluster.

$\bar{Y}_i$ = mean of the i-th cluster, and

$\bar{Y}$ = general mean.

The homogeneity index δ will be close to $+1$, if the between cluster variance accounts for a large part of the total variance and it will tend towards zero or sometimes negative (above the lower limit of it), if the between cluster variance accounts for a small part of the total variance. Clusters of moderately large size may be efficient sampling units if the δ-value is low positive (or negative) and less efficient when it is high positive. It is evident from Table I furnishing the values of δ in respect of the three types of clusters under examination, that for all types of clusters and borers as well the index is always very small indicating that there is heterogeneity within cluster which, in fact, is a strong support in favour of cluster sampling in this case. As the most moderate and suitable size for practical handling, the study has been confined to unequal clusters of 6′ row length.

TABLE I

Showing values of ' δ '

	Top borer			Stem borer			Root borer		
	3′ unit	6′ unit	9′ unit	3′ unit	6′ unit	9′ unit	3′ unit	6′ unit	9′ unit
Co 453 Plot I	0·0654	0·0546	0·0308	0·1238	0·1279	0·1092	0·0309	0·0990	0·037
Co 453 Plot II	0·0045	0·0343	0·0415	0·0208	0·0295	0·0147	0·0043	−0·0112	0·002
B.O. 11 Plot I	0·0577	0·0529	0·0435	0·0337	0·0518	0·0464	0·0182	0·0351	0·1511
B.O. 11 Plot II	0·0277	0·1088	0·0331	0·0119	0·0576	0·0503	−0·0276	−0·0056	0·0009

Efficiency of two sampling designs, namely, cluster sampling with subsampling, without and with stratification has been examined in this paper along with that of the following sample estimates:

(*a*) Mean of cluster means estimate $\bar{Y}_s$, given by,

$$\bar{Y}_s = \frac{1}{n} \sum^{n} \bar{Y}_i$$

and (*b*) Mean of cluster total estimate $\bar{Y}_s'$ given by

$$\bar{Y}_s' = \frac{1}{n\bar{M}} \sum^{n} M_i \bar{Y}_i$$

where,

$\bar{Y}_i$ is the sample mean of the *i*-th cluster,

M_i is the number of elements in the *i*-th cluster,

$\bar{M}$ is the mean number of elements per cluster, and

n is the number of clusters sampled.

The estimate in (*a*) is a biased but consistent estimate. Sukhatme[3] has shown that the magnitude of bias is small if the correlation coefficient between number of elements in the cluster and the cluster mean is small. Estimates of correlation coefficients have been presented in Table II.

TABLE II

Showing correlation coefficients between M_i and mean of clusters

Variety	Plot No.	Top	Stem	Root
Co. 453 ..	I	− 0·1006	− 0·0230	0·1258
Co. 453 ..	II	− 0·0036	− 0·0738	0·1697
B.O. 11 ..	I	0·3545	− 0·3897*	− 0·0361
B.O. 11 ..	II	− 0·0164	− 0·4021*	− 0·1168

* Indicates significance at 5% level.

An examination of the table will show that the coefficients are very small in about all the cases suggesting that this estimate, though biased, may be better than the unbiased estimate ($\bar{Y}_s'$) provided its sampling error is also comparatively less. For an examination of this point, thirty 6′-units were selected at random from the population of first stage units in a plot and from each of the selected units 4 random canes were selected. Also for examining

efficiency of stratification with rows constituting the strata, 5 random 6′ units were selected from each row with 4 random canes from each unit. Mathematical expressions of estimates of variances for these three cases are given below (after Sukhatme[3]).

(*i*) Variance of the mean of cluster mean estimate:

$$\text{Est. V}(\bar{\text{Y}}_s) = \left(\frac{1}{n} - \frac{1}{\text{N}}\right) s_b^2 + \frac{1}{n\text{N}} \sum^{n} \left(\frac{1}{m_i} - \frac{1}{\text{M}_i}\right) s_i^2.$$

Where,

$$s_b^2 = \sum^{n} \frac{(\bar{\text{Y}}_i - \bar{\text{Y}}_s)^2}{n-1}$$

$$s_i^2 = \frac{1}{m_i - 1} \sum_j (\text{Y}_{ij} - \bar{\text{Y}}_i)^2$$

N = Total number of 1st stage units in the population.

n = Total number of 1st stage units in the sample.

M_i = Number of 2nd stage units in the i-th 1st stage unit in the population.

m_i = Number of 2nd stage units selected as sample from the i-th 1st stage unit.

Y_{ij} = j-th element in the i-th 1st stage unit.

$\bar{\text{Y}}_i$ = Sample mean for the i-th 1st stage unit.

(*ii*) Variance of the mean of cluster total estimate:

$$\text{Est. V}(\bar{\text{Y}}_s') = \left(\frac{1}{n} - \frac{1}{\text{N}}\right) s_b'^2 + \frac{1}{n\text{N}} \sum^{n} \frac{\text{M}_i^2}{\text{M}^2} \left(\frac{1}{m_i} - \frac{1}{\text{M}_i}\right) s_i^2.$$

Where,

$$s_b'^2 = \frac{1}{n-1} \sum^{n} \left(\frac{\text{M}_i}{\bar{\text{M}}} \bar{\text{Y}}_i - \bar{\text{Y}}_s'\right)^2$$

$\bar{\text{M}}$ = average number of 2nd stage units per 1st stage unit and other symbols are same as in (*i*).

(*iii*) Variance of the unbiassed estimate in stratified two-stage sampling:

$$V(\bar{Y}_w)_s = \sum_t \lambda_t^2 \left\{ \left(\frac{1}{n_t} - \frac{1}{N_t} \right) S'^2_{tb} + \frac{1}{n_t N_t} \sum_i^{n_t} \frac{M_{ti}^2}{\bar{M}^2} \times \left(\frac{1}{m_{ti}} - \frac{1}{M_{ti}} \right) S^2_{ti} \right\}$$

Where,

$$\bar{Y}_w = \sum_t \lambda_t \bar{Y}'_{ts}; \quad \lambda_t = \frac{M_{t0}}{M_0}$$

$$= \frac{\text{Total number of 2nd stage units in the } t\text{-th stratum}}{\text{Total number of 2nd stage units in population}}$$

$\bar{Y}'_{ts}$ = Sample mean of t-th stratum

$$= \frac{1}{\bar{M}_t n_t} \sum_i^{n_t} \frac{M_{ti}}{m_{ti}} \sum^{m_{ti}} Y_{tij}$$

Estimates of S'^2_{tb} and S^2_{ti} are provided by,

$$\text{Est. } S'^2_{tb} = s'^2_{tb} - \frac{1}{n_t} \sum_i^{n_t} \frac{M^2_{ti}}{\bar{M}_t^2} \left(\frac{1}{m_{ti}} - \frac{1}{M_{ti}} \right) s^2_{ti}$$

and

$$s'^2_{tb} = \frac{1}{n_t - 1} \sum_i^{n_t} \frac{M_{ti}}{\bar{M}_t} (\bar{Y}_{ti} - \bar{Y}_w)^2$$

$$\text{Est. } S^2_{ti} = s^2_{ti} = \frac{1}{m_{ti} - 1} \sum^{m_{ti}} (Y_{ij} - \bar{Y}_{ti})^2.$$

M_{ti} = Number of 2nd stage units in the i-th 1st stage units of the t-th stratum.

m_{ti} = Number of 2nd stage selected units in the i-th 1st stage units of the t-th stratum.

Y_{tij} = j-th element in the i-th 1st stage unit of t-th stratum.

$\bar{Y}_{ti}$ = Sample mean of the i-th 1st stage unit of t-th stratum.

N_t = Number of 1st stage units in the t-th stratum.

n_t = Number of 1st stage units in the t-th stratum in sample.

$\bar{M}$ = Average number of 2nd stage units per 1st stage unit.

$\bar{M}_t$ = Average number of 2nd stage units per 1st stage in the t-th stratum.

The estimates of variances have been found out and standard errors expressed as percentages of respective means are presented in Table III.

TABLE III

Showing S.E. as percentage of mean

	Co. 453					
	Plot I			Plot II		
	$\bar{y}_s$	$\bar{y}_s'$	$(\bar{y}w)_s$	$\bar{y}_s$	$\bar{y}_s'$	$(\bar{y}w)_s$
Top ..	2·56	6·29	5·30	3·13	6·57	6·89
Stem ..	5·08	5·68	5·48	3·31	4·15	5·11
Root ..	3·54	5·93	6·25	3·36	6·64	7·07
	B.O. 11					
Top ..	3·56	6·85	7·12	2·87	5·86	5·63
Stem ..	4·11	5·76	5·88	3·31	4·00	3·76
Root ..	2·44	5·20	5·46	2·45	4·44	3·08

It is seen that in almost all the cases percentage errors of mean of cluster mean estimate ($\bar{y}_s$) are minimum suggesting that this estimate will be most suitable for all practical purposes. Relative efficiency of stratification is almost nil as will be evident from a scrutiny of Table IV furnishing these values.

TABLE IV

Showing per cent. efficiency of stratification

Variety	Plot No.	Top	Stem	Root
Co. 453 ..	I	141	107	90
Co. 453 ..	II	91	66	88
B.O. 11 ..	I	93	96	91
B.O. 11 ..	II	91	113	107

The probable sampling combinations of clusters × canes have been worked out for different values of m_i in respect of this estimate so that the error of estimation may not exceed 5% and 10% of the mean incidence of borers and are given in Table V. The figures in the parenthesis show the fraction of available canes to be sampled in order to get the estimated incidence at these levels of accuracy.

TABLE V

Showing hypothetical combination of canes × clusters

Variety Co. 453

Plot No. I

Total Number of canes = 1053

$\bar{M} = 14{\cdot}62$

	Error as 5% mean				Error as 10% mean			
	$m=2$	$m=3$	$m=4$	$m=5$	$m=2$	$m=3$	$m=4$	$m=5$
Top ..	41 (7·79)	35 (9·97)	33 (12·54)	32 (15·19)	12 (2·28)	12 (3·42)	11 (4·18)	11 (5·22)
Stem ..	53 (10·07)	43 (12·25)	39 (14·81)	37 (17·57)	15 (2·85)	14 (3·99)	14 (5·32)	13 (6·17)
Root ..	31 (5·89)	26 (7·41)	24 (9·12)	24 (11·40)	8 (1·52)	8 (2·28)	7 (2·65)	7 (3·32)

Co. 453

Plot No. II

Total number of canes = 871

$\bar{M} = 12{\cdot}10$

Top ..	27 (6·20)	22 (7·58)	21 (9·64)	20 (11·48)	7 (1·61)	6 (2·06)	6 (2·76)	6 (3·44)
Stem ..	35 (8·04)	27 (9·30)	24 (11·02)	23 (13·20)	8 (1·84)	7 (2·41)	7 (3·21)	7 (4·02)
Root ..	26 (5·97)	23 (7·82)	22 (10·10)	21 (12·06)	7 (1·61)	7 (2·41)	7 (3·21)	7 (4·02)

TABLE V (*Contd.*)

B.O. 11

Plot No. I

Total Number of canes = 891

$\bar{M} = 12{\cdot}38$

	Error as 5% mean				Error as 10% mean			
	$m=2$	$m=3$	$m=4$	$m=5$	$m=2$	$m=3$	$m=4$	$m=5$
Top ..	32 (7·18)	27 (9·09)	25 (11·22)	24 (13·47)	8 (1·80)	8 (2·69)	8 (3·59)	7 (3·93)
Stem ..	40 (8·98)	33 (11·11)	30 (13·47)	28 (15·71)	10 (2·24)	10 (3·36)	9 (4·04)	9 (5·05)
Root ..	23 (5·16)	18 (6·06)	16 (7·18)	15 (8·42)	5 (1·12)	5 (1·68)	4 (1·80)	4 (2·24)

B.O. 11

Plot No. II

Total Number of canes = 814

$\bar{M}=11{\cdot}31$

Top ..	20 (4·91)	17 (6·27)	16 (7·86)	15 (9·21)	5 (1·23)	5 (1·84)	5 (2·46)	5 (3·07)
Stem ..	33 (8·11)	26 (9·58)	23 (11·30)	22 (13·51)	7 (1·72)	7 (2·58)	7 (3·44)	7 (4·30)
Root ..	25 (6·14)	20 (7·37)	18 (8·85)	17 (10·44)	5 (1·23)	5 (1·84)	5 (2·46)	5 (3·07)

3. DISCUSSION OF RESULTS

Clusters of 3′, 6′ and 9′ row lengths have been found to be equally heterogeneous indicating suitability of cluster sampling in this case. Correlation coefficients between the number of canes in a cluster and mean value of the cluster are small and non-significant in all the cases except for stem borer incidence in both the plots under B.O. 11. The mean incidences of stem borer in these plots were also fairly high which suggest that for high levels of infestation, the unbiassed estimate, $\bar{y}_s'$, must invariably be taken

whereas for lower levels, the biased estimate $\bar{y}_s$ (arithmetic mean of cluster means) with minimum variance may be adopted, the magnitude of bias being very small. Stratification with rows as strata did not generally bring any improvement in the efficiency of the estimates. Sample sizes are highest for stem borer in all the plots pointing to heterogeneity in the distribution of incidence of this borer which had its effect on the variance function. Sampling fractions are minimum for root borer incidence. From the table furnishing sampling combinations it will be seen that a sample of 2 canes per cluster gives the minimum sample size in all the cases, thus providing optimum allocation within a cluster. It will be further seen that with this allocation a sample size not exceeding 10% of the available canes in the plot is needed for an estimate of incidence of all types of borers within an error margin of 5%.

4. SUMMARY OF RESULTS

(*i*) Efficiency of some sampling designs has been examined for estimation of incidence of sugarcane borers (Joint basis) in an experimental field.

(*ii*) Cluster sampling (6′ row length forming a cluster) with sub-sampling of canes in the cluster has been found to be the most suitable and efficient for the purpose.

(*iii*) Relative efficiency of stratification with rows as strata is nil in such cases.

(*iv*) The ordinary mean of cluster means, though biased, has the minimum variance and may be adopted only for lower levels of infestation, bias being negligible in these cases.

(*v*) A sample of 2 canes per cluster provides the optimum allocation and percentage total crop to be sampled with this allocation is about 10% for an estimate of incidence of all types of borers within an error margin of 5%.

5. ACKNOWLEDGEMENT

The work was carried out as part of the Sugarcane Research Scheme being financed jointly by the State Government of Bihar and the Indian Central Sugarcane Committee to whom grateful thanks are due.

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NEOTTIOSPORA CURCUMÆ SP. NOV.

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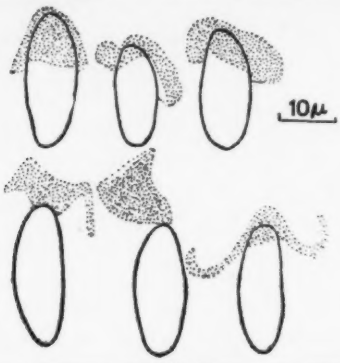
(Communicated by Prof. T. S. Sadasivan, F.A.SC.)

The fungus which forms the subject of this paper was collected on leaves of *Curcuma amada* Roxb. at Tambaram (Chingleput District, Madras State). It causes extensive drying of the leaves, sometimes involving the entire lamina. The pycnidia of the fungus are visible mostly on the upper surface of the leaves as very minute, black dots barely perceptible to the naked eye. They are ostiolate, more or less globose, immersed and occupying nearly half the width of the leaf tissue and slightly erumpent near the ostiole. The wall of the pycnidium is membranous and made of 2–3 layers of dark brown, pseudo-parenchymatous, polygonal cells. The conidia are produced all over the inner wall of the pycnidium. They are acrogenous on short, stout, somewhat clavate conidiophores. The conidia are thin-walled, hyaline, one-celled, cylindrical with rounded ends. Each conidium is provided with a mucoid, somewhat funnel-shaped appendage at its tip. This appendage is directed downwards in the earlier stages and forms a sort of cap which reaches half way down the length of the conidium. Various stages in the eversion of the appendage were observed. The appendage gets dissolved when the conidia are mounted in lactic acid but is clearly visible in aqueous mounts stained with methylene blue. It is also visible as a bright halo at or near the top of the conidium when it is mounted in dilute India ink. The development of the appendage seems to be as in *Neottiospora caricina* (Desm.) Hoehnel, as described by Subramaniam and Ramakrishnan (1953, 1957).

From the above description the fungus is easily identified as a species of *Neottiospora* Desm. Recent studies by Subramanian and Ramakrishnan (1953 and 1957) have clearly shown that the appendage in the genus *Neottiospora* is mucoid, evanescent and in the form of an inverted hollow cone. The description of the genus has therefore been emended by these authors so as to conform with this new interpretation of the spore appendage. Our fungus fits in very well with this emended concept of the genus. According to the same authors there are only three good species of *Neottiospora, i.e., N. caricina* (Desm.) Hoehnel, *N. paludosa* Sacc. and Fiori & *N. schizochlamys* Ferd. and Winge. We have compared our fungus with these three species. While the conidia of our fungus measure 16–25 × 5–8 μ, those of *N. caricina* are



TEXT-FIG. 1. *Neottiospora curcumæ*, conidia (from type, Herb. Mycol., No. 2857).

10–17 × 2–4 μ, those of *N. paludosa* are 24–49 × 4–8·5 μ and those of *N. schizochlamys* 23–39 × 5–8·3 μ. It is seen, therefore, that the conidia in our fungus are much larger than those of *N. caricina* and much smaller than those of the other two species. Moreover, while the three former species are saprophytic on dry leaves of members of the Cyperaceæ, the present fungus is parasitic on the leaves of a member of the Zingiberaceæ. It is, therefore, considered necessary to assign our fungus to a new species.

Neottiospora curcumæ *sp. nov.*

Foliicola, inducens dissecationem foliorum; pycnidia amphigena, ut plurimum epiphylla, minuta, nigra, immersa, tenuiter erumpentia, ostiolata, *plus* minusve globosa, 105–155 μ alta, 74–140 μ lata; parietes pycnidiales constant duplici vel triplici serie cellularum fusce brunnearum, pseudoparenchymaticarum; conidiophori emergunt ex pariete interno pycnidii, breves, robusti atque nonnihil clavati; conidia acrogena, unicellulata, tenuibus parietibus prædita, hyalina, cylindrica, apicubus rotundis, 19 × 6μ (16–25 × 5–8 μ), ornata appendice mucoidea apicali coni vacui inversi instar, 9–19 × 9–16 μ.

Typus lectus in foliis *Curcumæ amadæ* Roxb., in familia Zingiberacearum, in loco Tambaram (Chingleput District, Madras State) die 9 mensis februarii anni 1956 a N. V. Sundaram, et positus in Herb. Mycol. Gubernii, Coimbatore sub-No. 2857.

We are thankful to the Rev. Fr. Dr. H. Santapau, S.J., for having rendered the diagnosis into Latin.

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ERRATUM

Explanation of Plate IX facing page 94, *Vol. XLV* (2), *Section B, February* 1957

Circular paper chromatograms (*n*-Butanol/Acetic acid/Water:: 4: 1: 5 as solvent mixture) showing detection of gibberellic acid: (1) Pure gibberellic acid, (2) and (3) *In vivo* gibberellic acid in shoot and root respectively showing 'Bakanæ' symptoms.

SALINITY AND TEMPERATURE VARIATIONS IN THE SURFACE WATERS OF THE ARABIAN SEA OFF THE BOMBAY AND SAURASHTRA COASTS

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Received January 15, 1957

(Communicated by Dr. N. K. Panikkar)

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1. Introduction

The waters of the Arabian Sea extending from the West-Coast of India down to 100 fathoms and lying between 18° and 24° N. latitude are of great importance from the point of view of the fishing industry, as they support some of the richest trawling grounds off the Indian Coast. The exploitation of these grounds in recent years has yielded much valuable information on the nature and extent of the fisheries of these grounds, but unfortunately, we have no information on the hydrological and other environmental conditions. Though there have been a number of oceanographic expeditions in the equatorial areas of the Indian Ocean, very few stations have been worked in this region. The "John Murray" Expedition (1933–34) has carried out extensive observations—both biological and hydrographical—mainly along the Southern Coast of Arabia (Sewell, 1955). The study of the conditions of the waters of the Arabian Sea nearer the Indian Coast on the other hand has been much neglected in the past. What little information we have of this region is mostly derived from the observations made on the survey ships of the British Admiralty. The only detailed publication on the physico-chemical conditions of the waters of the Arabian Sea near Bombay was that of Bal *et al.* (1946), but the data are very much restricted in scope, their observations having been confined to the Bombay Harbour.

The present investigations were started during 1953–54 as part of a regular programme of offshore fishery investigations. In the early period, however, the observations were made mainly on the trawlers working from Bombay, and as the collection of water samples and other oceanographical data had to be adjusted to the fishing routine, it was not possible to make more than a few isolated observations. This is because the fishing operations were very much restricted to a few areas and also to a few months of the year. These preliminary observations have, however, given us some idea of the main hydrological trends on these grounds.

With the initiation of the oceanography scheme at the Central Marine Fisheries Research Station and the organisation of systematic collection of wate samples and other oceanographical data, more opportunities were afforded to combine the work on the fishing vessels with the collection of data on other coastal vessels, including those belonging to the Indian Navy. The present paper is an attempt to summarise the observations made so far on the surface temperature and salinity distributions in the Bombay and Saurashtra waters and to work out the main trends in the hydrological conditions in these waters.

2. MODE OF COLLECTION OF DATA

Ships belonging to different organisations and plying from Bombay were provided with water sample bottles, thermometers for determining surface sea-water temperatures and oceanographical log sheets (the forms having been standardised at the Central Marine Fisheries Research Station, Mandapam). Detailed written instructions were given to the masters of all the vessels for the collection of essential oceanographic data and water samples for analysis. Arrangements were made to take charge of the water samples, logs, etc., soon after each ship completed one voyage and touched port. By employing a large number of vessels for this work, gaps in the collection of data were avoided to a large extent, though not completely. The water samples were analysed for salinity soon after they reached the laboratory by the standard titration procedure using silver nitrate. The temperature and salinity for each station (Fig. 1) worked by the ships were tabulated and σ_t values were calculated. The data were grouped together in a manner similar to that described by Veen (1953) and the averages for each degree latitude* worked out.

* The data for each latitude were compiled by taking the values for stations half a degree on either side and averaging them. For example, the data for 19° N. represent the average of all stations between 18° 31′ and 19° 30′ N. latitudes, the data for 20° N. represent the average of all stations between 19° 31′ and 20° 30′ N. latitude and so on.

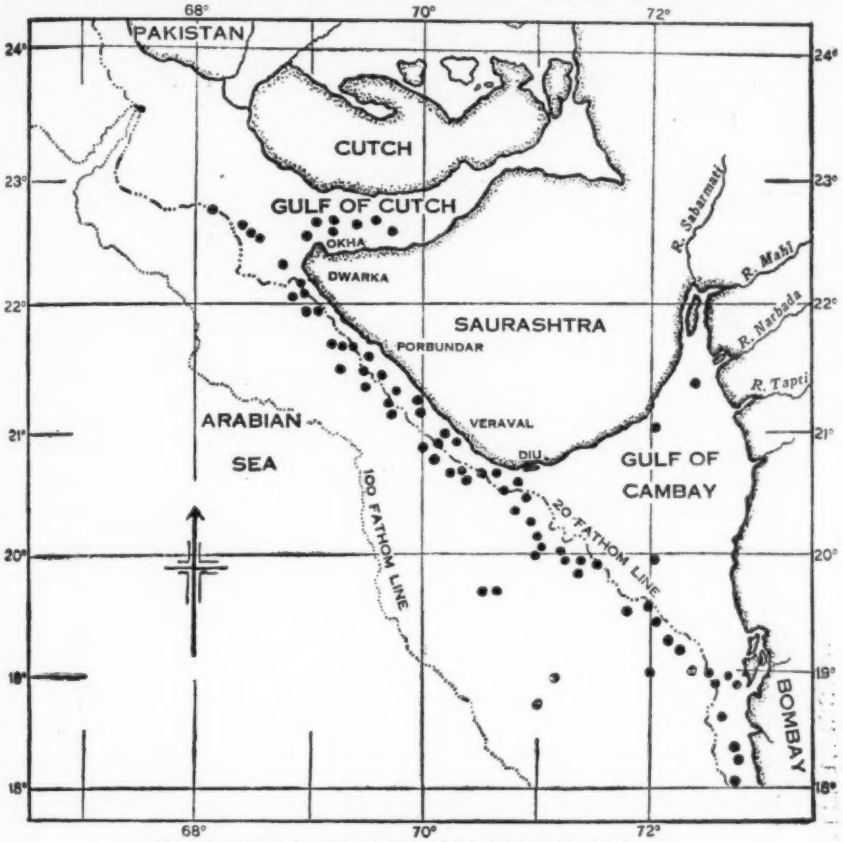

FIG. 1. Map showing the location of Sampling Stations.

3. RESULTS

The following tables show the mean monthly values for salinity, temperature and σ_t for each degree latitude between 18° and 23° N. latitude. The graphs (Figs. 2–6) give a clear picture of the seasonal trends in the different latitudes. For purposes of discussion, the conditions in each latitude are considered individually.

18° *N. Latitude.*—Data for this latitude are available only between October and March. It may be seen that the monthly range for surface salinities for the waters of this latitude is 33·65‰–36·13‰. The rise is sudden from October to November, after which the salinity remains steady upto January. The maximum is reached in February. After February, the salinity falls down by 1·28‰ to March.

TABLE I

Showing the mean monthly values for temperature, chlorinity, salinity and density of the surface waters of the Arabian Sea in latitude 18° N.

Months	Temperature °C.	Chlorinity Cl‰	Salinity S‰	Density σ_t
1955—				
October ..	26·8	18·62	33·65	21·79
November ..	28·3	19·77	35·71	22·96
December ..	25·7	19·85	35·86	23·83
1956—				
January ..	27·0	19·78	35·74	23·32
February ..	25·4	20·00	36·13	24·10
March	27·9	19·29	34·85	22·32

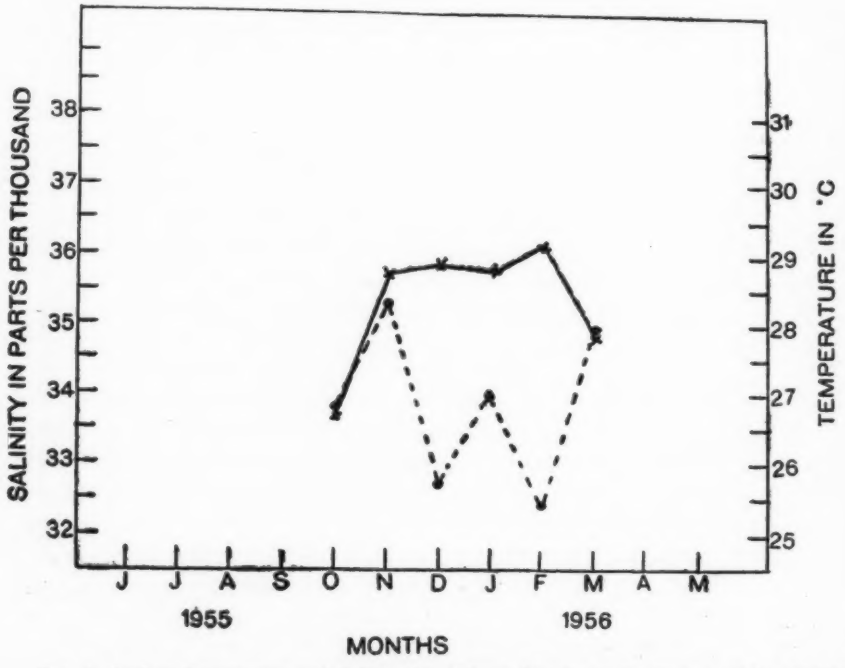

FIG. 2. Surface Temperature and Salinity in Different Months in the Waters of the Arabian Sea in Latitude 18° N. ×———× Salinity. ●·····● Temperature.

TABLE II

Showing the mean monthly values for temperature, chlorinity, salinity and density of the surface waters of the Arabian Sea in latitude 19° N.

Months	Temperature °C.	Chlorinity Cl‰	Salinity S‰	Density σ_t
1955—				
June	28·3	19·90	35·95	23·06
July	28·3	19·65	35·50	22·70
August	No data available for this month			
September ..	27·0	18·57	33·55	21·69
October	28·5	19·27	34·81	22·16
November ..	28·3	19·60	35·41	22·64
December ..	27·7	20·01	36·15	23·38
1956—				
January	25·9	19·85	35·87	23·77
February	25·2	19·74	35·67	23·82

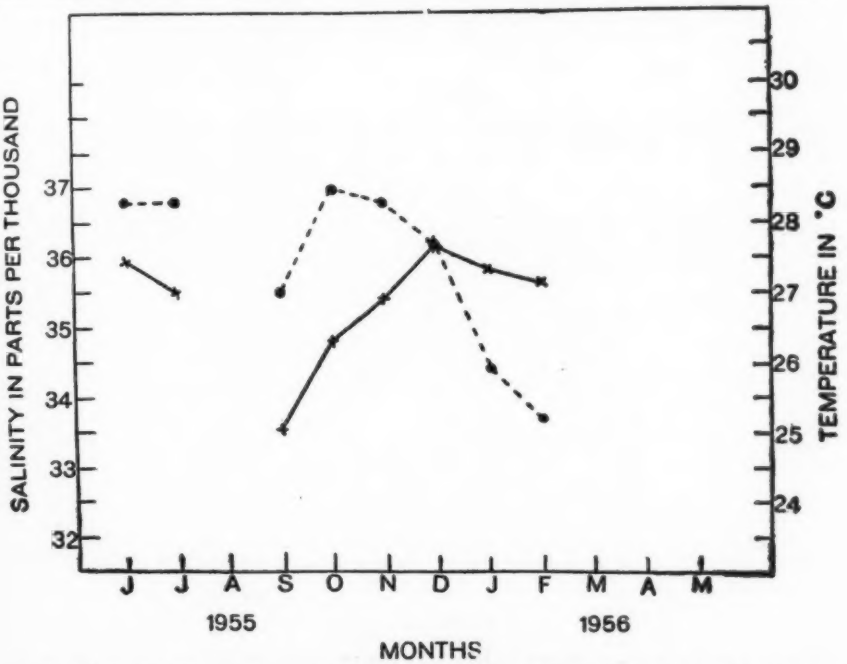

FIG. 3. Surface Temperature and Salinity in the Different Months in the Waters of the Arabian Sea in Latitude 19° N. ×———× Salinity. ●------● Temperature.

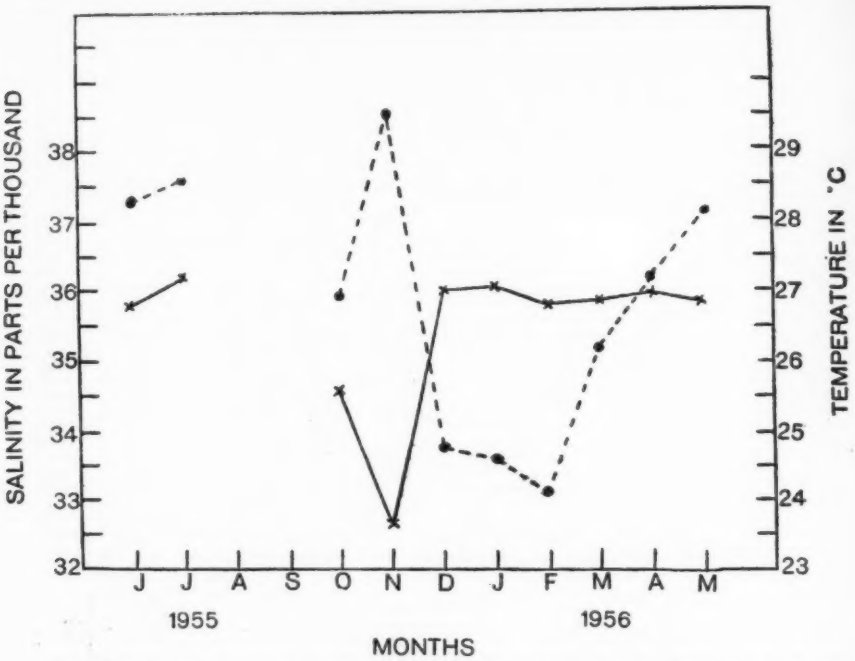

FIG. 4. Surface Temperature and Salinity in the Different Months in the Waters of the Arabian Sea in Latitude 20° N. ×———× Salinity. ●------● Temperature.

TABLE III

Showing the mean monthly values for temperature, chlorinity, salinity and density of the surface waters of the Arabian Sea in latitude, 20° N.

Months	Temperature °C.	Chlorinity Cl‰	Salinity S‰	Density σ_t
1955—				
June	28·3	19·80	35·77	22·92
July	28·6	20·04	36·20	23·11
August		Data not available		
September ..		Data not available		
October	26·9	19·14	34·58	22·49
November ..	29·5	18·07	32·65	19·28
December ..	24·7	19·94	36·02	24·26
1956—				
January	24·6	19·95	36·05	24·31
February	24·1	19·83	35·82	24·28
March	26·2	19·85	35·87	23·67
April	27·02	19·95	36·04	23·56
May	28·3	19·78	35·73	22·91

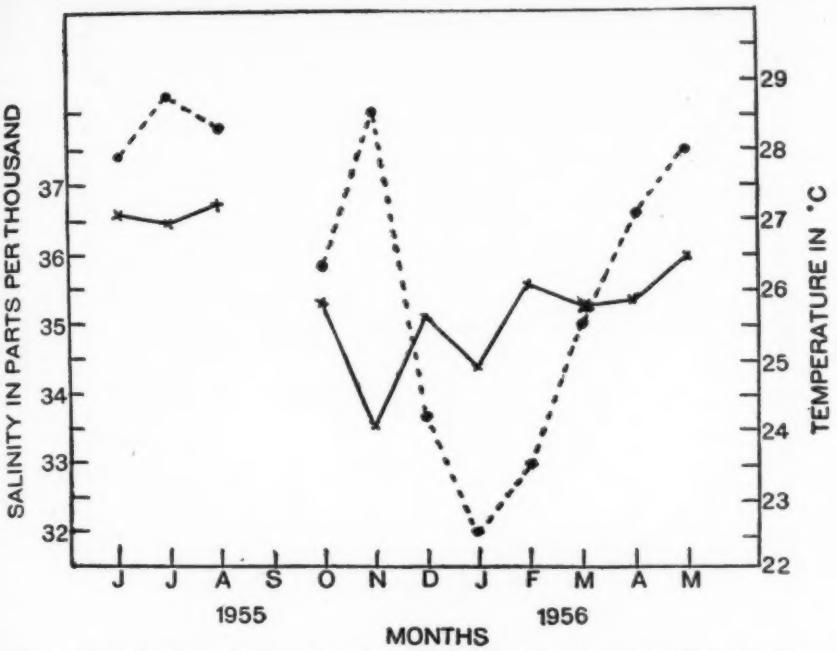

FIG. 5. Surface Temperature and Salinity in the Different Months in the Waters of the Arabian Sea in Latitude 21° N. ×———× Salinity. ●------● Temperature.

TABLE IV

Showing the mean monthly values for temperature, chlorinity, salinity and density of the surface waters of the Arabian Sea in latitude 21° N.

Months	Temperature °C.	Chlorinity Cl‰	Salinity S‰	Density σ_t
1955—				
June	27·9	20·26	36·60	23·70
July	28·8	20·18	36·45	23·24
August	28·3	20·35	36·76	23·66
September ..		Data not available		
October	26·3	19·54	35·30	23·19
November ..	28·5	18·57	33·55	21·21
December ..	24·3	19·44	35·11	23·64
1956—				
January	22·5	19·18	34·65	23·85
February	23·5	19·70	35·60	24·28
March	25·5	19·53	35·28	23·42
April	27·03	19·57	35·35	23·01
May	28·0	19·93	36·00	23·21

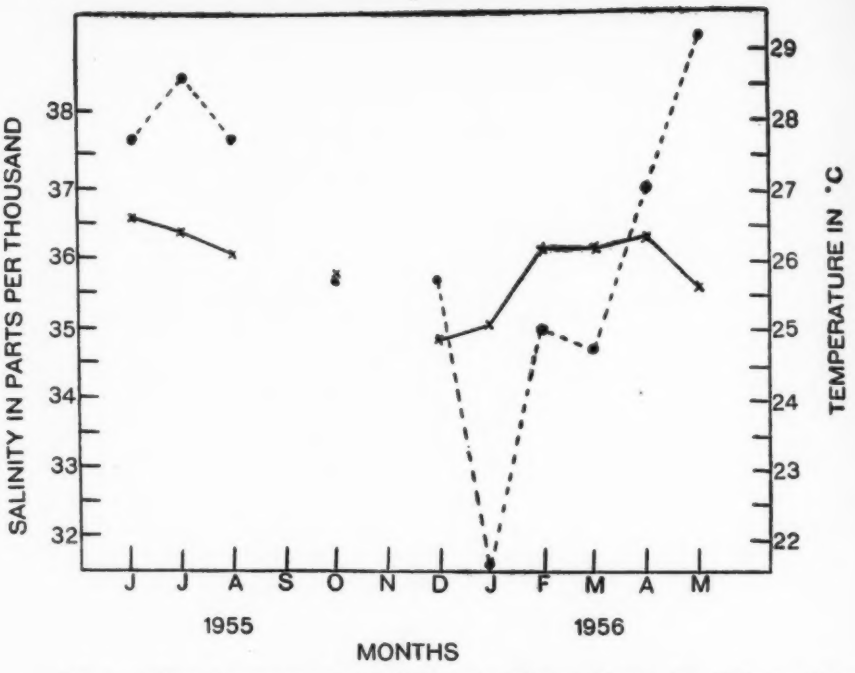

FIG. 6. Surface Temperature and Salinity in the Different Months in the Waters of the Sea in Latitude 22° N. ×———× Salinity. ●------● Temperature.

TABLE V

Showing the mean monthly values for temperature, chlorinity, salinity and density of the surface waters of the Arabian Sea in latitude 22° N.

Months	Temperature °C.	Chlorinity Cl‰	Salinity S‰	Density σ_t
1955—				
June	27·7	20·25	36·58	23·70
July	28·6	20·14	36·39	23·26
August	27·7	19·96	36·06	23·35
September ..		Data not available		
October	25·7	19·80	35·77	23·76
November ..		Data not available		
December ..	25·7	19·30	34·87	23·06
1956—				
January	21·6	19·43	35·10	24·42
February	25·0	20·02	36·17	24·25
March	24·7	20·01	36·15	24·30
April	27·0	20·12	36·35	23·74
May	29·2	19·67	35·53	22·44

TABLE VI

Showing the mean monthly values for temperature, chlorinity, salinity and density of the surface waters of the Arabian Sea in latitude 23° N.

Months	Temperature °C.	Chlorinity Cl‰	Salinity S‰	Density σ_t
1955—				
June	28·8	20·59	37·19	23·84
July	28·8	20·02	36·17	23·04
August		Data not available		
September ..		Data not available		
October		Data not available		
November ..		Data not available		
December ..	25·5	20·20	36·50	24·33
1956—				
January	21·0	20·27	36·62	25·74
February		Data not available		
March	25·0	20·49	37·01	24·90
April		Data not available		
May	28·5	20·13	36·36	23·27

In the case of surface temperature, there appears to be an alternation of high and low values. From 26·8° C. in the month of October, there is an increase to 28·3° C. in November, followed by a sudden fall to 25·7° C. in December. Again there is a rise to 27° C. in January, a fall to 25·4° C. in February, and then an increase to 28·0° C. in March.

19° *N. Latitude.*—During the months of the South-West monsoon, June and July, the surface temperature has been steady at 28·3° C., while the salinity has varied from 35·95‰ to 35·50‰. In the following period, the temperature has risen from 27° C. in September to 28·5° C. in October and then remained almost steady upto the end of November. The salinity has also shown an increase, the highest value being in December. The temperature, on the other hand, has recorded a fall by about 0·6° C. in December. In the months of January and February, both the salinity and temperature have shown a fall, the drop in the case of the latter being more sharp. The conditions in the subsequent months are not known, as the data for these months are not available.

20° *N. Latitude.*—In this region, which includes the waters of the Gulf of Cambay, the conditions during the South-West monsoon are almost similar to those observed in 19° N. Latitude. The trends are somewhat

different in the two areas in the months following the South-West monsoon. While the temperature continues to be high, the salinity has shown a marked decrease in the months of October and November, more particularly so in the latter. The temperature in November is as high as 29·5° C., probably the highest for the season, barring, of course, the summer months, while the salinity is as low as 32·65‰. In December the trend is completely reversed. The temperature has come down to 24·7° C. and the salinity has shown a sharp rise to 36·02‰. This change is seen to be very sudden. The high salinity persists thereafter till the end of May. The temperature, on the other hand, shows a further reduction to 24·1° C. in February and in March there is a sudden increase to 26·2° C.

21° *N. Latitude.*—The monthly trends are more or less the same as in the previous latitude. The salinity values during the South-West monsoon are somewhat higher. Between June and August, the salinity has varied from 36·45‰–36·76‰. Lowest values for salinity are reached in November, the mean value being 33·55‰. The sharp rise in salinity and drop in temperature are seen in December as in the previous case. But there are a few differences which deserve mention. The rise in salinity in December is slightly of a smaller order of magnitude than in latitude 20° N. Secondly, in the months following December, although a general tendency for higher values for salinity is seen, very often the hgih values are preceded and followed by slightly lower values. The increase in salinity thus proceeds in stages to the highest value of 36·00% in May. In the case of temperature, the initial lowering is similar to what has been seen in the latitude 20° N. The first lowering is from 28·5° C. in November to 24·3° C. in December. The reduction in temperature continues and the lowest value is reached in January, the mean temperature in this month being 22·5° C. A slight increase to 23·5° C. is observed in February, but in March the rise is sharp, the value being 25·5° C. It may, therefore, be seen that, barring the few differences mentioned above, the general pattern of salinity and temperature distribution is the same in the latitudes 20° and 21° N. The sudden changes noticed in these latitudes appear to be significant and will be discussed later.

22° *N. Latitude.*—The variations in salinity and temperature in this region show a somewhat different picture from what has been described before. The trend is a lowering of salinity from 36·60‰ in June to about 35‰ in December and January followed by a rise to about 36·17‰ in February and the steady maintenance of this value upto the end of April. In May the salinity has come down to 35·53‰. The temperature, on the other hand, shows a rather wide fluctuation. Between June and August, the mean values for temperature ranged between 27·7° C. and 28·6° C.

In October, the value has come down to 25·7° C. A decrease to 21·6° C. is seen in January, after which there is an increase to 25° C. which is followed by a slight drop to 24·7° C. The highest value for temperature 29·2° C. is reached in the month of May.

23° *N. Latitude.*—Very high salinities are found in the waters of this latitude—the average monthly salinity range being 36·17‰–37·19‰. The highest values have been observed in June and March of the following year. The temperatures are high in June and July and low from December onwards till next March. The value is again high in May—28·5° C. Unfortunately, the data for this region are rather scrappy and hence a continuous picture of the conditions has not been obtained. The lowest surface temperature has been obtained in January, the value being 21° C. The salinity remains practically unchanged, the mean annual range of variation being 1·02‰. The waters from inside the Gulf of Cutch seem to play a dominant role in affecting the hydrological conditions of these waters.

4. Discussion

The main factors controlling the distribution of surface temperature and surface salinity in Ocean waters have been discussed in great detail by Sverdrup *et al.* (1942). According to these authors, the surface salinity variations are determined by three processes:

(1) Decrease of salinity by precipitation;

(2) Increase of salinity by evaporation; and

(3) Change of salinity by processes of mixing.

These are probably the main factors affecting the distribution of surface salinity in the open oceans; whereas in coastal waters complications are introduced due to shifts in the coastal currents.

In the case of temperature, on the other hand, the controlling factors are a little more complicated. The annual variations in temperature are mainly due to the changes in the currents, vertical motion, seasonal heating and cooling and heat conduction.

In examining the present data with a view to explaining the observed variations in salinity and temperature, both in time and space, it would be necessary to have an idea of the prevalent current systems in these waters. A fairly detailed account of the currents in the Arabian Sea is given in the *West Coast of India Pi'ot*, published by the British Admiralty (1950) and also by Sewell (1955). It is seen that the main currents are those associated with the prevailing South-West and North-East monsoons, the speed and direction of these currents being regulated by the coastal confor-

mation. Besides these wind-driven currents, there are tidal streams of fairly high velocity, originating in the Gulf of Cambay and Gulf of Cutch, which play an equally significant, if not more important, part in governing the hyrdrological conditions of these waters.

During the months of the South-West monsoon, the general flow of the surface water is from West to East over the greater part of the Arabian Sea (Sewell, 1955). Due to the effect of the winds, the waters nearer our coast assume the character of the offshore waters, because of the " banking up " of the waters near the coast, sinking of these waters and replacement by more of the surface drift from the open sea. This may partly explain the prevalence of high salinities in these waters during the months of the South-West monsoon, in spite of the fact that the heavy rains during the season tend to dilute the surface waters, thereby lowering the surface salinity. The expected dilution effects of the rains are offset by the incursion of more saline waters of the open sea. The temperature, as could be seen, is also high during these months and this, again, could be ascribed to the incursion of the warm offshore waters.

October and early November form the transition between the cessation of the South-West monsoon and the commencement of the North-East monsoon. The winds are practically absent during this transition period. In October, the surface waters get heated to a considerable extent, which may explain the prevalence of high surface temperatures in this month and in early November also. There should be a certain amount of evaporation during these months, but this probably is countered by the influx of fresh-water. This is particularly noticeable in the Gulf of Cambay and a little to the North of it upto 21° N. latitude. The lowest salinities observed in the months of October and November are, therefore, to be attributed to dilution caused by the mixing of large volumes of fresh-water from the rivers like Narbada, Tapti, etc., which open out into the Gulf of Cambay (see Fig. 1). It is interesting to note that such marked lowering in salinity has not been noticed in the waters further North—around 22° N. latitude. This is mainly due to the absence of any major rivers in the vicinity of this latitude.

From the latter half of November, there is a change in the conditions. The predominant winds are from the North and North-East. With the onset of these winds, the pattern of currents also changes. It is a well-known fact that owing to the combined action of the wind and the effect of rotation of the earth, the surface waters of the oceans in the northern hemisphere will be forced in a direction of about 45 degrees to the right of the wind direction. Thus it may be seen that during this season, the surface waters tend

to be taken more and more away from our coast. During the same season, Sewell (1955) has reported that on the East African and Arabian Coasts, the surface water tends to be " banked up " near the coast. As a consequence of these water movements, nearer our coast a certain amount of upwelling from moderate depths will be taking place to replace the water driven seawards, the intensity as well as the extent of this upwelling being determined by the force and duration of the wind. It may be seen that the hydrological conditions of these waters, particularly around 20°-21° N. latitude and to a slightly less extent in 22° N. latitude, undergo a sudden change immediately after November. The high surface temperature and low salinity of the October-November period give place to low temperature and high salinity almost within a few days. Such a condition persists from December to almost the end of February. It is quite possible that this type of change in the conditions is due to the upwelling of the coastal waters, which has resulted in the replacement of the exisiting body of water with a different one from below. The present data may appear insufficient to establish this conclusively but the indications are fairly clear.

Upwelling of the waters along the East Coast of India has been reported by La Fond (1954, 1955) and along the East African and Arabian Coasts by Sewell (1955). From an examination of vertical temperature sections and seasonal surface temperature and salinity cycles, La Fond has shown that upwelling takes place along the Visakhapatnam Coast between March and April when the prevailing winds are from the South-West. It could be seen that in those months the trend of surface water is seaward, almost similar to what has been indicated by us along this coast between December and February.

The intensity of the northerly winds begins to decrease from the beginning of March. The summer heating of the surface waters takes place and the surface temperature begins to increase. The coastal circulation is reversed in direction—instead of the waters flowing away from the coast, the flow is towards the coast. The force of the onshore current increases as South-West monsoon season approachs. A general rise in surface salinity of these waters is seen in the months following March. This rise in salinity is partly due to evaporation and partly due to admixture with the high salinity water, from the Persian Gulf brought in by the onshore currents during this period. The prevalence of high salinity may be explained as being due to the operation of these factors.

It could thus be seen that the hydrological conditions of these waters are very much influenced by the prevailing seasons, which can be described

as Pre-South-West monsoon, South-West monsoon, Transition, and North-East monsoon.

We are thankful to Dr. N. K. Panikkar, Chief Research Officer, Central Marine Fisheries Research Station, Mandapam, for his keen interest and encouragement in the course of these investigations, and in the preparation of this paper for publicaiton.

5. SUMMARY

The distribution of surface temperature and salinity in the waters of the Arabian Sea along the Bombay and Saurashtra coasts is presented and discussed.

The two factors show seasonal trends associated with the prevalence of the two monsoons, South-West and North-East.

In general, waters North of 20° N. latitude have a higher salinity and lower temperature than waters South of it.

The variations in salinity and temperature indicate the possibility of seasonal upwelling in the coastal waters between 20° and 21° N. latitude and to a less extent upto 22° N. latitude during the period December to February.

It is suggested that these mixing phenomena may be of great importance in determining the fertility of these waters.

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AN INVESTIGATION OF LARGE-SCALE FISH MORTALITY IN A TEMPLE TANK IN MADURAI*

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Introduction

During the last decade, some cases of large-scale fish mortalities have been investigated in the waters of Madras State by Chacko and Ganapathi (1949), Chacko and Srinivasan (1954 *a* and *b*), Ganapathi *et al.* (1950) and Venkataraman *et al.* (1956). Fish mortalities have been reported from other parts of our country also (Alikunhi *et al.*, 1951). An annual occurrence of the fish mortality has been reported in Uttar Pradesh by Moitra (1955). Similar phenomena have also been recorded by Sewell (1927) and Pruthi (1932) in Indian waters. Whipple *et al.* (1927) have discussed about the sudden mortalities of a number of fishes in the lake at Weequabic Reservation, U.S.A.

Although this phenomenon is of frequent occurrence in our State resulting in a considerable loss of fish food supply of the country, the timely prevention of such calamities, at least in the Departmental waters, is of great significance. This is possible only when one knows the physical, chemical and biological factors that are responsible for the phenomenon. With this object in view, a case of large-scale fish mortality that occurred in the Mariamman Teppakulam, Madurai (South India) in November 1955, was investigated and is reported in this communication.

History of the Occurrence of Mortality

It was gathered from local enquiries that the phenomenon of mortality is an annual feature in this tank, though on a minor scale, culminating into a major one on rare occasions. The large-scale mortality of fish that is being reviewed now appears to have taken place concurrently on 2 or 3 days, between 18th and 20th of November 1955, though the mortality was actually reported to have started in the course of the first week of November, 1955. It was reported that about two tons of decaying fish were removed from the tank and destroyed by the Public Health Staff of Madurai Municipality.

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DESCRIPTION OF THE TANK

The Mariamman Teppakulam is a square tank of 17 acres. The sides are paved with stones with a number of steps alround. The tank has a maximum depth of 16′ during the rainy season and 5′ during the summer months. There is an artificial island in the centre which is provided with a summer house. The tank was constructed during the period of the Naick Kings of Madura, about the 17th Century. It is now used only for the " Floating Festival " of the local Deity of the Madura Meenakshi Temple. A large number of pilgrims and local populace use the tank for bathing, washing of clothes and utensils, etc. Originally a channel from river Vaigai was feeding the tank. Now the channel is in ruins by disuse and river water does not find access to the tank. Hence at present the tank is purely rain-fed. The storm waters of the town also get into the tank through two inlets, during the rains. The main channel on the North Western corner of the tank crosses over a sewage drain about 200 yards from the tank. The channel enters a crude sort of silting tank (which is also now in disuse) and divides into two, one entering the tank at the North-West corner and the other taking off towards the South and entering the tank at the South-West corner.

HYDROBIOLOGICAL CONDITIONS

During the time of inspection there was water to a depth of 11′ in the Teppakulam. The bottom was found to be uniform with black oozy silt to a depth of about 6″. A sample of the bottom silt was analysed and the details are given in Table I. Samples of water were collected from both surface and bottom on 22nd and 23rd November 1955. The bottom samples of water were collected with a Freidinger deep water sampler, operated from a boat. The results of analysis are detailed in Table II.

TABLE I

Showing the chemical conditions of the bottom ooze of Mariamman Teppakulam, Madurai in November, 1955

1.	Carbonates	..	Present in moderate quantities
2.	Nitrates	..	5·0 p p.m.
3.	Ammonia	..	Less than 2 p.p.m.
4.	Phospherous	..	1·25 p.p.m.
5.	Calcium	..	100·0 p.p.m.
6.	Magnesium	..	More than 6 p.p.m.
7.	Iron ..	..	3·0 p.p.m.
8.	Nitrites ..	..	Less than 1·0 p.p.m.
9.	Chlorides	..	50·0 p.p.m.
10.	Sulphate	..	150·0 p.p.m.
11.	pH ..	..	More than 8·0

TABLE V. *Showing the chemical conditions in Mariamman Teppakulam on 22nd and 23rd November 1955*

		22–11–1955				23–11–1955			
		Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom
1.	Time of collection	7–00 A.M.	7–30 A.M.	4·10 P.M.	4–30 P.M.	7–00 A.M.	7–30 A.M.	4–00 P.M.	4–30 P.M.
2.	Depth ..	Surface	11 feet	Surface	11 feet	Surface	11 feet	Surface	11 feet
3.	Met. data ..	Bright sunlight and windy				Intermittent cloudy windy			
4.	Temperature °C.	29·4	29·2	30·3	29·5	29·6	2?·4	29·8	29·4
5.	Colour ..	Dirty green in appearance as a mass and pale yellow when collected in bottle							
6.	Odour ..	Putrefying smell of decaying organic matter							
7.	Turbidity (in cm.)	22	18	21·0	18·0	22·0	19·0	21·0	19·0
8.	Dissolved O_2 Mg./l.	1·5	1·0	3·4	1·5	1·5	0·8	3·0	1·3
9.	c.c./l. ..	1·047	0·698	2·373	1·047	1·047	0·558	2·94	0·987
10.	%	19%	12·5%	42·8%	18·7%	18·7%	9·9%	52·6%	17·5%
11.	Free CO_2 p.p.m.	Nil	5·5	Nil	5·5	Nil	2·2	Nil	2·2
12.	Carbonates p.p.m.	6·0	Nil	42·0	Nil	3·0	Nil	36·6	Nil
13.	Bicarbonates ..	369·05	381·25	274·5	396·5	372·1	378·2	347·7	356·85
14.	Chlorides p.p.m.	390·0	400	380	390	380	380	380	390
15.	pH	8·7	8·4	8·5	8·5	8·5	8·4	8·5	8·5
16.	Silicates p.p.m. ..	8·0	8·0	5·0	6·0	5·0	8·0	6·0	8·0
17.	Phosphates ..	0·54	0·55	0·5	0·5	0·5	0·6	0·6	0·55
18.	Total hardness ..	168	170	165	173	169	170	164	167
19.	Free Chlorine p.p.m. ..	Nil							
20.	Nitrous Nitrogen p.p.m. ..	Trace							
21.	Nitric Nitrogen p.p.m. ..	Trace							
22.	Free Ammonia p.p.m. ..	0·88							
23.	Albuminoid Ammonia ..	0·44							
24.	B.O.D. (3 days) p.p.m. ..	5·00							
25.	Tidys (Oxygen absorbed from $KMnO_4$) p.p.m.	6·666							
26.	Total solids p.p.m.	1460·0							
27.	Organic matter p.p.m. ..	900·0							
28.	Electric Conductivity (at 20° C.)	1350							
29.	Hydrogen Sulphide	Present in moderate quantities							

N.B.—Tests (19) to (29) were carried out for only one sample.

The water of the Teppakulam was dirty green in colour and teamed with multitudes of Zoo plankton, *Tilapia* fry and suspended matter. The marginal waters also exhibited an oily film at the surface. An offensive fishy odour of dead and decaying organic matter pervaded the tank. Large bubbles of gas were seen rising to the surface and gettting collected, before finally breaking up. This occurred at the shore, where the high stony banks afforded shelter to the decaying algal scum and decaying floating mass of vegetable matter. When the bottom silt was disturbed, bubbles of gas were seen rising to the surface in rapid succession in large quantities bringing up particles of dark floating debris. At the time of our investigation, stray cases of dead and decaying fish were noticed floating here and there. Fishes were also found coming up to the surface in groups and gasping for breath.

Plankton

Five collections were made for the study of the plankton by filtering 10 litres of the water through a townet of 178 mesh bolting silk. The analysis was carried out by the method described by Chacko and Krishnamurthi (1954), and the number of plankters per litre is given in Table III.

Fish Fauna

The following species and numbers of fish had been stocked in the tank continuously from 1949 onwards:

Chanos chanos	..	1,91,5089
Catla, Labeo spp. *and Cirrhina* spp.	..	44,6156
Etroplus suratensis	..	13,293
Tilapia mosambica	..	23,504
Mirror Carp and Golden Carp	..	5,174

DISCUSSION

The dirty green appearance of the water mass is the result of the abundance of suspended matter and rich planktonic growth, coupled with humus. The sample collected in bottles had a yellowish hue as described by Ruttner (1953) in connection with bog waters. The secondary colour maxima in ponds, *i.e.*, in November and December described by Welch (1935) seems to be in concurrence with the dirty green waters of the Teppakulam found during our visit in November.

The offensive odour of the waters was due to the large quantities of organic matter, decaying algal scum and other organisms including the dead and decaying fish coupled with the presence of hydrogen sulphide. Such evidence of foul odours are possible due to pond disturbance together with the presence of free ammonia (Thresh *et al.*, 1949).

TABLE III

Showing the No. of plankters per litre of water in Mariamman Teppakulam on 22nd November 1955

Organisms	Eastern Portion	Southern Portion	Western Portion	Northern Portion	Deep Water (10 feet)
Zooplankton					
Brachionus	1,08,000	2,08,000	2,24,000	4,83,000	..
Diaphanosoma	2,00,000	80,000	..	2,31,000	12,000
Bosmina ..	6,52,000	6,50,000	3,26,000	1,26,000	..
Macrothrix..	36,000	33,000	1,68,000	8,40,000	24,000
Nauplii ..	18,000	..	..	42,000	12,000
Cypris ..	..	8,000	..	..	..
Mosquito larvæ	1,000	..	..	..	..
Total ..	10,15,000	9,79,000	7,18,000	17,22,000	48,000
Phytoplankton					
Cyanophyceæ					
Merismopedia	..	1,00,000	1,00,000	..	1,50,000
Spirulina ..	50,000	50,000	17,00,000	..	..
Chlorophyceæ					
Pediastrum	..	..	50,000	..	..
Scenedesmus	10,000	50,000	6,00,000	1,00,000	1,00,000
Desmidiaceæ					
Staurastrum	..	1,00,000	..	1,00,000	..
Bacillariaceæ					
Melosira ..	..	..	..	..	5,00,000
Nitszchia ..	5,50,000	2,50,000	15,00,000	50,000	3,00,000
Navicula ..	8,00,000	6,50,000	115,00,000	50,000	..
Euglenids					
Euglena ..	..	..	50,000	..	1,00,000
Total ..	14,10,000	12,00,000	115,00,000	3,00,000	11,50,000

The variations between the surface and the bottom temperatures are very low especially on 23rd (Table II). The reason for the comparatively negligible variations between the morning and evening temperatures of the surface and the bottom may be due to the fact that the day was practically cloudy and the solar radiation comparatively less. The tank abounding in floating debris and planktonic growth has been behaving thermally as turbid waters, the surface waters absorbing the heat readily and rapidly transmitting to deeper layers, as pointed out by Forell (1895). The atmospheric temperature on 22nd was 81° F. (27·2° C.) and on 23rd it was 80·5° F. (26·9° C.). Thus both the surface and bottom waters of the tank registered higher temperatures than air temperatures.

According to Atkins and Harris (1924) waters with a pH of over 8·1 are certainly indicative of supersaturation of oxygen. But such supersaturation of oxygen was not met with in the Mariamman Teppakulam, where dissolved oxygen was poor in general both at the surface and bottom. The low oxygen may be partially due to the rapid consumption of the gas by the large number of plankters and other organisms and greater depletion being due to the decomposition of the organic matter at the bottom making greater demands on the available oxygen, including the direct consumption of the gas by bacterial activity. Welch (*loc. cit.*) has observed that complete exhaustion of dissolved oxygen may occur owing to the combined consumption activities of animals, plants and bottom deposits and such extreme reduction of oxygen is more common than is realised. Hence it may not be strange to assume that such a contingency might have actually occurred during the period of mortality.

There is a close relationship between insufficient dissolved oxygen and diseases of fish and serious cases of diseases and mortality occur. According to Welch (*loc. cit.*) such natural tragedies are common in artificial ponds having no drainage and containing unusual organic deposits as is the case with the Mariamman Teppakulam. The low dissolved oxygen content and its consequent insufficiency for respiration at the lower depths and other inimical conditions have driven out the gasping fish population in the tank to the surface waters on 22nd and 23rd, as also observed by Ganapathi *et al.* (*loc. cit.*). The complete absence of carbon dioxide at the surface and presence at the bottom layers is noteworthy. Its absence in the surface may be due to its utilisation by the rich phytoplankton for their phytosynthetic activity and also due to wave agitation. Powers (1937) feels that such difference in CO_2 tension between the bottom and surface brings about a physiological derangement and sudden mortality of fish.

The bicarbonates were present both at the surface and bo tom on an average from 340·89 to 378·2 p.p.m. respectively. The quantity of bicarbonates is slightly greater in the bottom samples than at the surface, due to the fact that free carbon dioxide was sufficiently available at lower depths converting all the available carbonates into bicarbonates, while the lower quantity at the surface was due to the precipitation of carbonates (Ruttner, 1953).

According to Thresh *et al.* (*loc. cit.*) any excess of ammonia over 0·1 p.p.m. in combination with excess of organic matter is clearly indicative of manurial pollution and any excess of ammonia over 0·04 p.p.m. can be construed as derived from sewage pollution. Thus sewage pollution in Mariamman Teppakulam is strongly indicated by free ammonia figure of 0·88 p.p.m. The high albuminoid ammonia figure in the Teppakulam waters indicates large quantities of organic matter in solution (Drew, 1914).

Nitrates are derived mainly from the organic matter of animal pollution and to some extent by oxydation of vegetable matter. But only traces of nitrates and nitrites are found during the formation or reduction of processes. According to Thresh *et al.* (*loc. cit.*) in the absence of air and in the presence of nitrites, the animal matter undergo decomposition and consequent reduction and disappearance of nitrate. Hutchinson *et al.* (1932) have reported mere traces of and total absence of nitrates in South African lakes and ponds. This is supported by Beadle (1934) who has found similar phenomena in the Rift valley lakes of East Africa.

The phenomena of the rising of bubbles noticed in the tank may be partially due to the accumulated carbon dioxide formed by decomposition of the bottom deposits rising up due to internal pressure (Welch, *loc. cit.*). It may also comprise of bubbles of hydrogen sulphide and methane. A similar case of gases escaping from the bottom has been described by Ganapathi *et al.* (*loc. cit.*) . The presence of hydrogen sulphide gas has been detected in Mariamman Teppakulam waters, qualitatively. It is possible that the sulphides of soil by bacterial reduction aided by the organic decay might have been responsible for the production of hydrogen sulphide. The black iron sulphide might have been precipitated in the presence of hydrogen sulphide in the alkaline reaction of the waters, the iron again going into the cycle of oxydation reduction. Catastrophic mortalities of fish are possible during turn overs due to the presence of hydrogen sulphide, according to Ruttner (*loc. cit.*).

Taking 40·0 p.p.m. of chlorine in chlorides as being normal (Thresh *et al.*, *loc. cit.*), the amount may increase to 200 upwards, the increase

accompanied by an increase of the ammonia and organic matter. Sewage as a rule contains 50·0 p.p.m. of chlorine in chlorides than that contained in normal waters. Thus a chloride figure of 380 to 400 p.p.m. found in the Mariamman Teppakulam waters is derivative from no other source than sewage pollution particularly of animal origin as described by Ganapathi (1950).

The B.O.D. (3 days test) of the tank waters was 5·0 p.p.m. It therefore comes under the classification of waters of doubtful purity (Thresh *et al.*, *loc. cit.*). The Tidy's value of over 6·0 p.p.m. as evidenced in the case of Mariamman Teppakulam waters should be considered with suspicion confirming the indication of sewage pollution inferred earlier.

Plankton was comparatively more abundant in the surface waters. The presence of Euglenoids again indicated pollution. The authors in the course of a study on the production of plankton through aquatic manuring have had occasion to observe multitudes of Euglenoids alone developing in cement nurseries with crude sewage and grass that were absent in the control. The presence in large numbers of diatoms in the Teppakulam is also indicative of pollution (Needham and Lloyd, 1937). Needham and Lloyd (*loc. cit.*) have also associated the presence of *Scenedesmus* with stagnant water conditions, especially polluted ones. The zoo-plankton was represented mainly by Daphnids. They were present only in the surface waters of the tank. This may probably be due to the presence of rich algal food at the surface as compared to the bottom waters, and due to the development of inimical conditions at the bottom through chemical changes forcing the zoo-plankton to the top layers (Welch, *loc. cit.*).

CONCLUSION

The Mariamman Teppakulam is rain-fed and receives drainage waters including the town sewage. The absence of any outlets brings on stagnation, which has been taking place over several years. A glance at the Meteorological data (Table IV) of the town will show that in the first fortnight of November, there had been a number of rainy days, especially on 12th and 13th. During the heavy rains in the first week of the month the town drainage with the crude sewage from the overflowing sewage drain flooded the tank upsetting the balance in the tank and welling up harmful decomposing gases, depleting the dissolved oxygen content to lethal limits, and the gradual rendering of the waters inimical to aquatic life, especially considering the large number of fish that have been stocked in the tank. Fowler (1944) states that the Manchester Ship Canal which gets occasionally polluted with sewer and storm waters, becomes incapable of supporting fish life.

TABLE IV

Showing meteorological data of Madurai Town from 1st November 1955 to 23rd November 1955

Date November 1955	Temperature Max.	Temperature Min.	Humidity %	Rainfall in inches Past 24 hours	Rainfall total since 1-11-1955	Remarks
1st	90	74	74	Traces	30·2	Rain
2nd	90	70	85	1·7	31·9	
3rd	86	71	88	0·4	32·3	Rain
4th	82	74	87	Nil	32·3	Nil
5th	88	70	93	1·0	33·3	
6th	85	74	85	Traces	33·3	
7th	91	71	75	Nil	..	
8th	93	73	68	..	..	
9th	92	70	67	..	..	
10th	91	70	69	..	..	
11th	89	70	67	..	..	
12th	9	73	79	..	..	
13th	89	70	93	1·4	34·7	Rain
14th	87	70	69	Traces	34·7	Rain
15th	87	73	70	..	..	
16th	87	72	62	..	..	
17th	87	71	70	..	..	
18th	88	71	79	..	..	
19th	89	75	79	..	..	
20th	87	75	79	..	..	
21st	87	73	79	..	..	
22nd	89	73	74	Traces	34·7	Rain past 24 hours
23rd	88	73	79	0·4	35·1	Thunder showers past 24 hours

Continued display of unfavourable conditions have had a progressively deleterious effect on the biota of the water, gradually weakening them and creating a susceptibility to sudden mortalities. The most significant factor was the heavy rains on the 13th, further upsetting the inimical conditions in existence. In addition to the direct effect of depletion of dissolved oxygen, other conditions have also contributed to the mo·tality.

No single factor can therefore be isolated and stated as the chief cause of the phenomenon. But each one of the factors discussed above in detail have individually and collectively proved lethal to the fish. Again it cannot be construed that the mortality had occurred on any particular date only, as

it was likely that the fishes as they were dying would have been settling at the bottom for some time before floating up at the surface in a decomposed state.

SUMMARY

The large-scale fish mortality that occurred in the Madurai Mariamman Teppakulam in November, 1955, is discussed in detail. The tank had been accumulating all kinds of organic debris, including periodic pollution by sewage. Consequently the waters have been rendered inimical to life contained in it. The meteorological conditions that prevailed during the period has culminated in the upwelling of all poisonous gases from the tank bottom proving fatal to fish life.

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NOTES ON SOME FUNGI

By T. S. Ramakrishnan, F.A.Sc.

Received March 22, 1957

In December, 1956, I visited Ranchi in Bihar and Hirakud in Orissa. Among the collections of fungi made during my stay there some were new and others not previously recorded from these localities. These are described in this communication.

Phyllactinia heterophragmitis sp. nov.

Mildew growth hypophyllous, forming effuse white patches or sometimes covering the whole surface of the leaflets; conidia broadly clavate, apex obtuse, hyaline, 36–52 × 16–25 μ. Perithecia numerous, dark brown, globose, sometimes depressed, 185–200 μ in diameter, with an equatorial ring of appendages 14–18 in number, appendages 140–215 μ long, bulbous base 40 μ broad. Most of the perithecia were immature.

Hypophylla, efformans maculas albas effusas, vel aliquando totam superficiem foliorum cooperiens; conidia late clavata, apice obtuso, hyalina, 36–52 × 16–25 μ. Perithecia plura, fusce brunnea, globosa, nonnumquam depressa, 185–200 μ diam., ornata annulo aequatoriali appendicum 14–18; appendices vero 140–215 μ longæ, bulbousaæ ad basim 40 μ latim. Peritheciorum plurima immatura.

On living leaves of *Heterophragma roxburghii* DC. (Bignoniaceæ), Namkum (Bihar), 22-12-1956, T.S.R.

The mildew was noticed on most of the leaves. The upper surface of the leaflets in the affected portions was yellowish in colour. The perithecia were numerous and varied in colour according to age from yellow to orange or reddish brown. The bulbous base of the appendage was thin-walled while the upper portion was either thin or thick-walled.

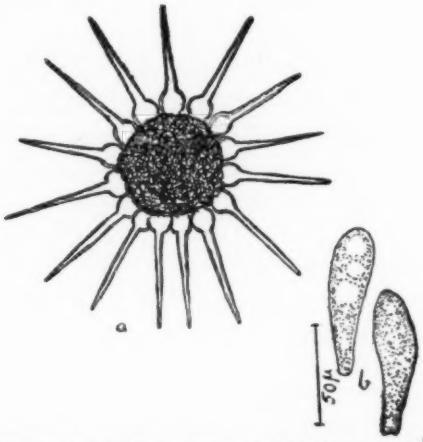
Phyllactinia terminaliæ sp. nov.

Hypophyllous, mycelium sparse or thick, in white patches of different sizes or covering the whole surface. Conidia hyaline, broadly clavate, rounded at the apex, rounded or truncate at the base, 45–60 × 15–24 μ. Perithecia scattered on the lower surface, honey coloured to dark brown according to maturity, 170–200 μ in diameter, globose or spherical, appendages in a ring of 16–18 structures, hyaline, 120–210 μ long, with a bulbous base.

Penicillate cells numerous. Asci brief pedicellate, 2–3 spored, with yellowish contents, 50–60 × 24–28 μ.

Hypophylla, mycelium sparsum vel densum, in maculas albas magnitudinis variæ vel in totam paginam dispersum. Conidia hyalina, late clavata, ad apicem rotundata, ad basim rotundata vel truncata, 45–60 × 15–24 μ. Perithecia dispersa per inferiorem paginam, mellea vel fusce brunnea colore, pro statu ad maturtatem, 170–200 μ diam., globosa vel spherica. Appendices 16 vel 18, structurarum in annulum coactæ, hyalinæ, 120–210 μ longæ, basi bulbosa. Cellulæ penicillatæ plures. Asci breviter pedicellati, 2–3 spori, contentis luteis, 50–70 × 24–28 μ.

On living leaves of *Terminalia chebula* Retz. (Combretaceæ), Ranchi (Bihar), 20–12–1956, T.S.R.



TEXT-FIG. 1. *Phyllactinia terminaliæ* sp. nov.—(*a*) Perithecium (enlarged). (*b*) Conidia.

This tree is common here and is sometimes planted in the road avenues. Most of the trees were infected in December. Tender leaves were free while the older ones carried the mildew. Since the mycelium penetrated the mesophyll, the infected portion could be recognised from the upper surface by the yellow colour, owing to loss of chlorophyll. Numerous perithecia of different ages could be seen scattered on the lower surface. The youngest ones were almost yellow while others were orange, honey coloured or dark brown.

Phyllachora malloti sp. nov.

Leaf-spots of different shapes. Stromata amphigenous, round or irregular, one or more in a spot, black, raised, shining, with mammiform projections on the surface, multiloculate; asci numerous, hyaline, cylindrical, shortly

stipitate, apex rounded, 100–125 × 8–10 μ, paraphysate; ascospores 8, monostichous, oval to spindle-shaped, 9–12 × 4·5–6 μ, hyaline.

Maculæ foliorum figuras variabilis. Stromata amphigena, rotundata vel irregularia, singula vel plura in singulis maculis, elevata, nitentia, pr jectionibus mammiformibus ad superficiem, multiloculata; asci plures, hyalini, cylindrici, breviter stipitati, ad apicem rotundati, 100–125 × 8–10 μ, paraphysati; ascosporæ 8, monostichæ, ovales vel fusiformes, 9–12 × 4·5–6 μ, hyalinæ.

On living leaves of *Mallotus* sp. (Euphorbiaceæ), Namkum (Bihar), 24–12–1956, T.S.R.

The leaves were covered with numerous tar spots. The stromata were formed in light green necrotic areas.

Masseella putranjivæ sp. nov.

Pycnia minute, reddish brown, subcuticular, amphigenous, often closely associated with æcia, 70–95 × 34–46 μ.

Aecia hypophyllous, in groups, subepidermal, 250–300 × 100–170 μ; æciospores catenulate, hyaline, oval oblong or subglobose, minutely verrucose, 17–21 × 11–14 μ, Uredia mostly hypophyllous, white, in groups or solitary, subepidermal, erumpent, powdery; urediospores hyaline, elliptical to subglobose, 18 × 14 μ, echinulate.

Telia epiphyllous, reddish brown, hair-like, curved or coiled, 1·5–2 mm. long and 0·1–0·2 mm. thick, subepidermal in origin; teliospores oval or subglobose, dark brown, smooth, one-celled, pointed or rounded at the tip, wall thickened up to 2·5 μ, embedded in a colourless gelatinous matrix, 21–33 × 14–19 μ, with a prominent germ pore.

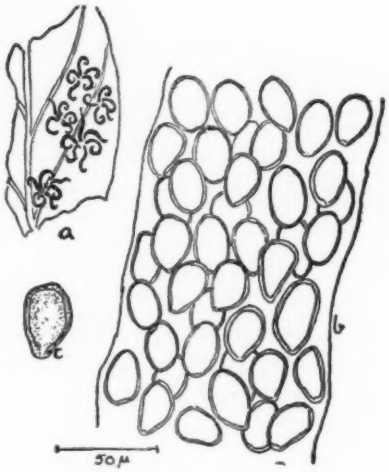
Pycnia minuta, rubro-brunnea, subcuticularia, amphigena, sæpe in æcia associata, 70–95 × 34–46 μ.

Aecia hypophylla, aggregata, subepidermalia, 250–300 × 100–170 μ; æciosporæ catenulatæ, hyalinæ, ovatæ oblongæ vel subglobosæ, minute verrucosæ, 17–21 × 11–14 μ. Uredia ut plurimum hypophylla, alba, solitaria vel aggregata, subepidermalia, erumpentia, pulverulenta; urediosporæ hyalinæ, ellipticæ, vel subglobosæ, 18 × 14 μ echinulatæ.

Telia ut plurimum epiphylla, rubrobrunneis, capillaceis, curvati vel circumvolutis, 1·5–2 × 0·1–0·2 mm., subepidermalibus origine. Teliosporæ ovatæ, ellipticæ vel subglobosæ, fusce brunneæ, leves, semel-cellulatæ, acutæ vel rotundatæ ad apicem, parietibus crassis usque ad 2·5 μ, infixæ in matricem

haud coloratum gelatinosum 21–33 × 14–19 μ, germinationis poro unico eminente.

On living leaves of *Putranjiva roxburghii* Wall. (Euphorbiaceæ), Ranchi (Bihar), 24–12–1956, T.S.R.



TEXT-FIG. 2. *Masseella putranjivæ* sp. nov. (*a*) A bit of leaf with telia (enlarged). (*b*) Bit of telial filament. (*c*) One teliospore.

Severe infection of all the plants in the area had occurred. The upper surface of the leaves had become reddish brown owing to the presence of numerous telial filaments. The æcia were empty in some instances and appeared as open clusters of cups. Peridia were not prominent. Uredia were often mealy with the whitish powdery urediospores strewn about in the neighbourhood.

Ravenelia clemensæ Syd.

Sydow, H. and Petrak, F, *Ann. Mycol.*, **20**, 418, 1928.

On leaves of *Albizzia* sp. Hirakud Dam (Orissa), 12–12–1956, T.S.R.

This rust was prevalent to a moderate extent on some of the trees planted in the avenue. Both the uredial and telial stages were present.

Uredo tephrosiicola P. Henn.

Sydow, H. and P. .. *Mongr. Uredin*, 1923–24, **4**, 485.

McRae, W. .. Rept. of the Imperial Mycologist in *Repts. of the Agric. Res. Inst. and Coll. Pusa*, 1927–28, **69**, 1928.

On living leaves of *Tephrosia candida* DC. (Papilionaceæ), Ranchi (Bihar), 15–12–1956, T.S.R.

This rust was prevalent on almost the entire crop of this plant grown by the Forest Department on a hill in the city. McRae had recorded this rust on the same host but the exact locality has not been given in the Fungi of India by Butler and Bisby.

Cercospora zinniæ Ellis and Martin

Ellis, J. B. and Martin .. *J. Mycol.*, 1885, **1**, 20.

Chupp, C. .. *Monograph on the Fungus Cercospora*, 1953, p. 168.

On living leaves of *Zinnia elegans* (Compositæ), Hirakud Dam (Orissa), 12–12–1956, T.S.R.

This leaf-spot affected most of the plants in a bed. The spots were circular to irregular with the centre whitish to grey and margin reddish brown. The conidiophores were mostly epiphyllous. The conidia were hyaline and acicular and multiseptate.

I am indebted to Sri. J. Sakkarama Rao of the Agricultural College, Coimbatore, for the identification of some of the host plants. My grateful thanks to Rev. Fr. H. Santhapau, S.J. of the St. Xavier's College, Bombay, for having kindly translated the diagnoses into Latin.

CONTRIBUTION TO OUR KNOWLEDGE OF THE PHYSIOLOGICAL ANATOMY OF SOME INDIAN HYDROPHYTES

VI. The Stem of *Lobelia alsinoides* Lam.

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Received February 5, 1957

(Communicated by Dr. T. S. Sabnis, F.A.SC.)

Introduction

Lobelia alsinoides Lam. is a small annual herb which is suberect, forming many spreading branches from its base. It thrives in damp places and on the shallow margins of lakes and ponds. The genus *Lobelia* Linn. is the only hydrophytic genus of the family Lobeliaceae (sensu stricto) growing in our country where it is represented by about fifteen herbaceous species, some of which grow in damp places and marshy areas.

The stem of *Lobelia alsinoides* Lam. is slender and glabrous. Its upper portion is acutely three-angled, often forming wing-like projections as in *L. trialata* Ham. The lower portion, which may be submerged for some time, shows a more or less circular outline, the characteristic wings of the upper portion being absent here (*cf.* Figs. 1 and 2).

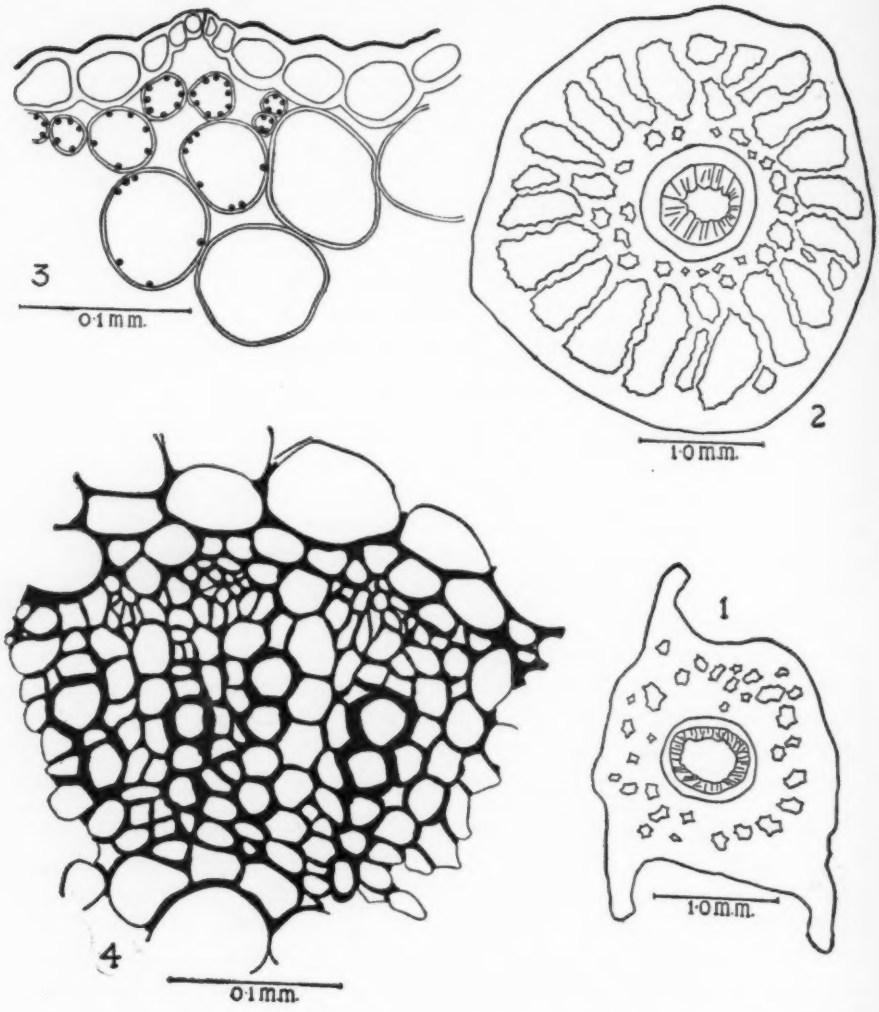
Material and Method

The material was collected from the wet banks of the Pingala Talao at Umred, about twenty-nine miles to the south-east of Nagpur, as well as from the damp bottoms of annual puddles at Mansar, about twenty-two miles to the north-east of Nagpur. It was fixed in formalin acetic alcohol. The anatomical description is based on the study of free-hand sections of the stem taken at different levels. The Text-Figs. represent camera-lucida drawings made by the author.

Description

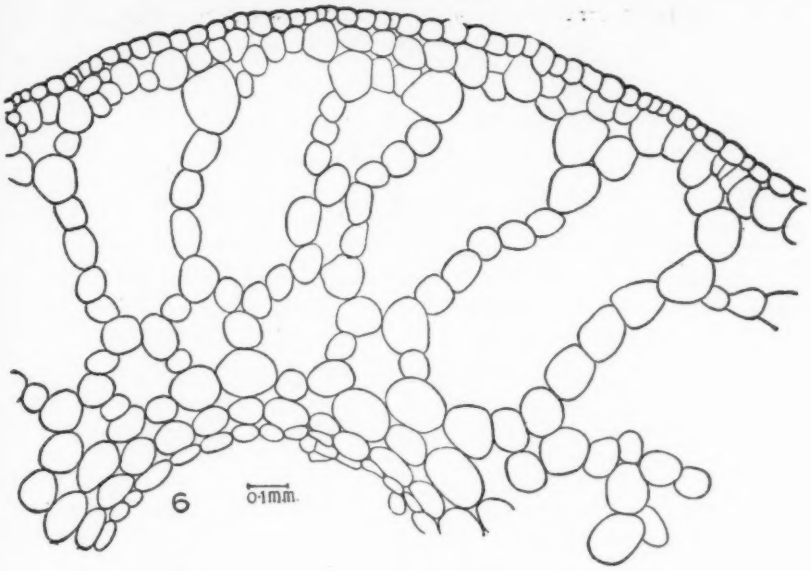
1. *Epidermis.*—Outer walls thickened; cuticle very thin; anthocyanin occasionally present. Stomata raised (Fig. 3).

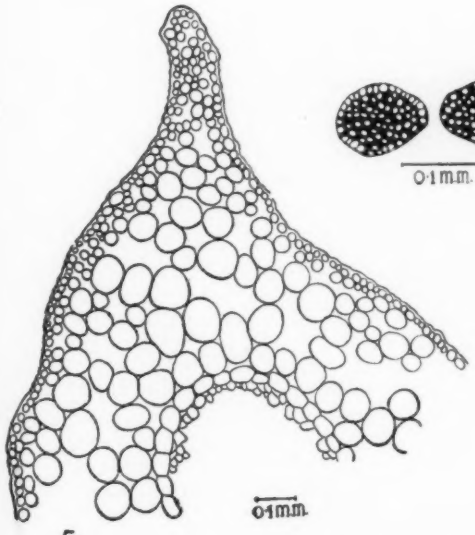
2. *Cortex.*—Outer cortex of two to three layers of chlorenchyma; collencyma entirely wanting. Wings in the upper portion of the stem entirely chlorenchymatous. Middle cortex lacunar; lacunæ smaller and fewer in

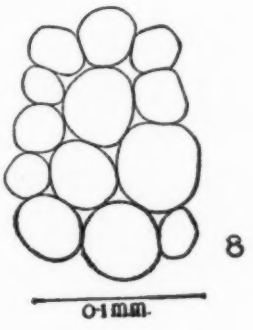


TEXT-FIGS. 1–4. *Lobelia alsinoides* Lam. Fig. 1. T.S. of the upper portion of the stem (semi-diagrammatic). Fig. 2. T.S. of the lower portion of the stem (semi-diagrammatic). Fig. 3. Part of the T.S. of the stem showing the raised stoma, the thin cuticle, the thick-walled epidermal cells and the chlorenchymatous thin-walled cells of the outer cortex. Fig. 4. Part of the T.S. of the stem showing the pericycle, phloem and xylem.

the upper portion, large and abundant in the lower (*cf.* Figs. 1 and 2 and 5 and 6). The thin-walled parenchymatous cells that line the prominent schizogenous lacunæ show the development of pitted plates on their walls (Fig. 7).







TEXT-FIGS. 5–8. *Lobelia alsinoides* Lam. Fig. 5. Part of the T.S. upper portion of the stem showing the poorly developed lacunæ of the middle cortex. Fig. 6. Part of the T.S. of the lower portion of the stem showing the pronounced development of the lacunæ and the elongation of the thin-walled parenchymatous cells of the middle cortex. Fig. 7. Two pitted cell plates on the walls of the cortical cells (surface view). Fig. 8. Part of the T.S. of the stem showing the thin-walled parenchymatous cells of the narrow pith.

3. *Pericycle*.—One to two layered, slightly thick walled (Fig. 4).

4. *Vascular system* (Fig. 4).—Phloem and xylem form a closed cylinder. The xylem vessels are arranged in well-defined radial rows. Phloem is traversed by a number of laticiferous vessels.

5. *Pith*.—Narrow, thin-walled parenchymatous (Fig. 8).

DISCUSSION

1. The genus *Lobelia* Linn. includes both arborescent as well as herbaceous species. The arborescent forms, which occur in the mountainous regions of tropical and sub-tropical Africa, have been the subject of investigation by a number of workers. Armand (1912) has worked on the herbaceous, submerged species, *L. dortmanna* Linn., which inhabits the margins of lakes in North Europe and North America. As far as the author is aware, his is the first account of the physiological anatomy of the wetland species of the genus from India.

2. As in the case of the stems of most hydrophytes, investigated earlier by the author, the aerating system is provided by the middle cortex which is made up of thin-walled, parenchymatous cells enclosing a number of schizogenous lacunæ. In *Lobelia dortmanna* Linn. also it is the primary lacunar cortex that serves for aeration (Armand, 1912). The lower portion of the stem, which may be submerged, or situated in a soil saturated with water, shows a pronounced development of this tissue. The expansion of the cortex consequent on the enlargement of the lacunæ and the elongation of the cortical cells naturally leads to an apparent disappearance of the wing-like projections in the lower part of the stem. There is, thus, no actual loss of the vegetative tissue of the wings as in the stem of *Sphæranthus indicus* Linn. (Mirashi, 1956). This type of variation in the extent and elaboration of the cortical aerating system in the upper and lower portions of the stem is characteristic of the marsh plants. It has been observed earlier in the stems of *Eclipta alba* Hassk. (Mirashi, 1955) and *Sphæranthus indicus* Linn. (Mirashi, 1956). The expansion of intercellular spaces may be the mechanical result of the pressure of gases evolved, which are prevented by the surrounding water from escaping freely, as suggested by Goebel (1891–93). But, it may also have a relation to the difficulty of breathing in water, or in a soil saturated with water, and the consequent need for the storage of larger quantities of air, resulting in the elaboration of the aerating tissue.

3. A feature hitherto unobserved in the stems of the hydrophytes investigated earlier, is the development of pitted plates on the walls of the parenchymatous cells that constitute the lacunar cortex. These plates

serve for mechanical rigidity; for, in the anatomy of this stem, as in the stem of the species of *Lindernia* Allioni (Mirashi, 1957), the fragile lacunar cortex is strengthened neither by the usual peripheral shell of collenchymatous outer coretex nor by means of diaphragms. Such plates have not been reported so far to occur in the cortical cells of the stems of any species of *Lobelia* Linn., although Armand (1912) has found them between the cortical cells of the root of *L. dortmanna* Linn.

ACKNOWLEDGEMENT

The author wishes to express his deep sense of gratitude to Dr. T. S. Sabnis, D.SC., F.A.SC., for kindly going through the manuscript of this paper.

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THE NUCLEUS OF THE LIVING ZYGOTE OF *SACCHAROMYCES CARLSBERGENSIS*

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Introduction

The recent attempts to elucidate the structure of the resting nucleus of yeast using the living cell as a "standard" (Royan and Subramaniam, 1956; Royan, 1956 *a*, *b*, *c*; Muller, 1956; Thyagarajan and Subramaniam, 1957) originate from the general dissatisfaction felt by investigators of the limitations of orthodox fixing and staining procedures when applied to yeast. The invisibility of the nucleus under most physiological conditions had precluded so far an accurate evaluation of the reliability of the various cytological procedures for a study of the resting nucleus.

To fill up this important lacuna in our knowledge, a programme of active search for the living nucleus was initiated in this laboratory three years back and cultures of various species and strains grown under differing physiological conditions were examined systematically with a phase contrast microscope. The nucleus was visible in 40–60% of the cells from 96–120-hour wort cultures of a strain of *Saccharomyces cerevisiæ* grown at 24–26° C. Micrographs taken under bright field, phase contrast and dark ground illumination (Royan and Subramaniam, 1956; Royan, 1956 *a*, *b*, *c*) were presented and it was shown that the nucleus could be kept under observation during the cytological procedures like fixation, hydrolysis and staining (Royan, 1956 *a*). A fair idea of the reaction of the nucleus to a variety of fixatives and stains is now available (Royan, unpublished).

In *Saccharomyces carlsbergensis* the nucleus could be seen in 5–10% of the living cells from 160–190-hour wort cultures (Thyagarajan and Subramaniam, 1957). In *S. cerevisiæ* as well as in *S. carlsbergensis* the nucleus if once located under phase contrast could be observed under ordinary illumination. Muller (1956) studied the role of the refractive index of the media in rendering visible the nucleus in yeast cells in which it is normally invisible. A comparison of the photomicrographs presented by Muller (1956) of cells of *S. carlsbergensis* mounted in a medium of suitable refractive index, with those obtained by us (Thyagarajan and Subramaniam, 1957) revealed that more nuclear details could be observed in 7-day wort cultures.

There are no previous reports on the nucleus of living zygotes of yeast. The zygote of *Saccharomyces carlsbergensis* possesses a very desirable characteristic. The shape assumed at the time of its origin by fusion of two spores is retained even after the zygote had produced a few buds. Since the nucleus becomes visible in the vegetative cells only when the culture has aged, the possibility of the nucleus being visible in those zygotes retaining their characteristic shape 6–7 days after the introduction of the spores into fresh wort was explored. The nucleus is clearly visible in 40–50% of the zygotes in 7-day cultures.

HISTORICAL RESUMÉ

Reports on the zygote nuclei based on stained preparations are rather few. From material fixed in Perenyi's fluid and stained with hæmatoxylin, Guilliermond describes the zygote nuclei of *Saccharomyces ellipsoideus* var. Johannisberg II (1910) and *Schizosaccharomyces octosporus* (1917) as vesicular and possessing a nuclear membrane, a nucleolus and often a chromatic reticulum.

Winge (1935) employed two different procedures for the study of the zygote nuclei of *Saccharomyces ellipsoideus* var. Johannisberg. For staining with hæmatoxylin the fixation was in Muller's fluid, while for the Feulgen technique it was in chloroform. The nucleus described and illustrated by him is a solid structure lacking the finer details reported by Guilliermond (1910).

Badian (1937) using the Giemsa stain observed two chromosomes in the spores as well as the zygotes of *Saccharomyces cerevisiæ*. He did not observe a nuclear membrane.

Lietz (1951) does not refer to the existence of a nuclear membrane in zygote nuclei though he records its presence in vegetative cells. Widra and DeLamater (1955) investigated *Schizosaccharomyces octosporus* but their description of the zygote nucleus is at variance with that of Guilliermond (1917). They surmise the existence of a nuclear membrane even though they could not reveal the nuclear details illustrated by Guilliermond.

EXPERIMENTAL PROCEDURES

We are indebted to Dr. L. J. Wickerham for the gift of the strain of *Saccharomyces carlsbergensis*. Cells from 16-hour barley malt wort (S.G. 1·020; pH 4·6–4·8) cultures were streaked on wort agar slopes. When growth had proceeded for 24 hours material was transferred to acetate agar (Sodium acetate 0·4%; Agar 1·5%, *cf.* Fowell, 1952) slants and examined periodically. Spores begin to appear 24–36 hours after transfer to acetate

and since 10-day old slants contained a large number of spores, they were used as the source for the germination studies.

Not all cells sporulate on acetate agar and naturally the inoculum introduced into wort would contain vegetative cells also. These were killed by keeping the tubes in water at 55° C. for five minutes. Due to the heat treatment zygotes begin to appear only after 16 hours after transfer to wort and viable spores give rise to zygotes within 48 hours.

When the nuclei become visible in the zygotes on the 7th day, the cells were mounted in a drop of the medium in which they had grown and two moist filter-paper strips were kept on either side of the square coverslip to flatten the cells uniformly and prevent their movement. They were photographed with a Leica attachment on Kodak ' Microfile ' film. The negatives were later enlarged for reproduction.

Observations

1. *Living Cells*

Photo 1 is that of a zygote observed three days after the introduction of the spores into wort. The cytoplasm appears dense, the two vacuoles are clear, but the nucleus cannot be located in the living cell. It would seem that any region of a zygote can produce a bud (Photos 1–3 and 5–10). In Photos 9 and 10 the bud has originated from the bridge-like waist of the zygote. Attention is invited to the zygote in micrograph 5 which has two buds of differing ages still attached to it (compare with Fig. 9 A, Lietz, 1951).

With the aging of the culture the cytoplasm slowly loses its opacity under phase contrast and the nucleus becomes gradually visible (compare Photo 1 with Photos 5–10). On the 7th day it could be photographed in favourable examples even under ordinary illumination (Photos 2, 3 and 4). It has a conventional structure in that the nuclear membrane (NM, Photos 2, 3 and 4) which delimits it from the cytoplasm encloses visible structures (Royan and Subramaniam, 1956; Royan, 1956 *a*). A crescentic denser area attached to the inner surface of the nuclear membrane is visible in Photo 3 while a grain is present in Photo 4.

The phase contrast microscope reveals more details. The structure of the nucleus shows considerable variation. The nucleus in Photo 6 has the appearance of a dense ring enclosing in its clear interior a distinct grain. The thickness of the nuclear periphery is not uniform in the zygote illustrated in micrograph 7. As in the previous instance there is a distinct granule in the middle of the clear area. That the thickened appearance of the nuclear periphery is due to formed structures plastered on to the inner surface of the

nuclear membrane would become evident from Photo 8. The nuclear membrane (NM) is thin near the clear interior of the nucleus. Half the nucleus is partially opaque (Photo 8) reminding one of the crescentic veil-like area in micrograph 3. There is a dense sickle-shaped mass plastered on to the inner surface of the nuclear membrane. A grain is lying free inside the nucleus.

Since the zygotes had buds attached to them, the culture was scanned for instances where the nuclei could be observed simultaneously in a zygote and its bud. Micrographs 9 and 10 illustrate such a rare example. The two nuclei were not at the same optical plane and hence two photographs are presented to illustrate their orientation as well as structure. The cytoplasm was optically denser than in the cells shown in Photos 6, 7 and 8 with the consequence that the nucleus does not show good contrast as in the preceding illustrations. The nucleus of the bud has the same structure as that of the zygote. What is more, it resembles in structure the living nucleus of the vegetative cells illustrated earlier (Thyagarajan and Subramaniam, 1957).

2. *Fixed and Stained Preparations*

Two investigators (Guilliermond, 1910; Winge, 1935) who studied the nucleus of *Saccharomyces ellipsoideus* var. Johannisberg have presented differing descriptions of the structure of the nucleus. The details observable in the nucleus of the living zygotes, illustrated by photographs for the first time in this paper, if used as a basis for an evaluation of the published results would suggest that the fixation and staining procedures employed by Guilliermond gave a picture approaching that of the living nucleus. Guilliermond's suggestion that the nuclei of the zygotes and vegetative cells resemble each other in that they are delimited from the cytoplasm by a nuclear membrane is borne out by micrographs 9 and 10 illustrating such a membrane in the living nuclei of a zygote and its bud (*cf.* Royan, 1956 *a*, *b*, *c*; Thyagarajan and Subramaniam, 1957).

The earlier descriptions of nuclear structure (Guilliermond, 1910; Winge, 1935) are based on fixed and stained preparations owing to the invisibility of the nucleus in the living zygote. Naturally later investigators agree with Guilliermond or Winge depending on the technique employed and the results obtained by them (Ganesan, 1956). The way one handles the staining procedures becomes the criterion for evaluation in the absence of a " standard " to judge as to how far the stained preparations resemble living structure. It is in this context that the discovery of the nucleus in a living zygote is interesting. An accurate evaluation of the effects of fixatives and stains on the living nucleus of yeast is now available (Royan, 1956 *a*, and unpublished observations).

Considerable care has to be exercised in the selection of the standard. According to Wager (1898), Nageli and Henneguy had observed the nucleus in living yeast cells with an ordinary microscope. Guilliermond's (1910) comment that the nuclei of zygotes and vegetative cells show an identity in structure in stained preparations necessitates the selection as a standard a living zygote nucleus having a nuclear membrane enclosing formed structures inside.

Because the living nucleus is visible only under particular physiological conditions in some strains of yeasts (Royan and Subramaniam, 1956; Royan, 1956 *a*, *b*, *c*; Thyagarajan and Subramaniam, 1957) the easy availability of the phase contrast microscopes did not produce any spectacular advances in yeast cytology. According to Mundkur (1954) and Muller (1956) the nucleus appears often only as a homogeneous body under the phase contrast type of illumination. If such a living structure is taken as the standard, then, the stained nucleus should appear as a ' solid ' body and structural details if revealed by fixatives and stains are likely to be considered as artefacts.

The function of a good fixative is to produce a life-like preservation of the cell organelles. Since the living nucleus is taken as the standard, the fixative should preserve not only the nuclear structure but also that of the vacuole. Iodine-formaldehyde-acetic acid mixture was the fixative of choice in view of Royan's (1956 *a*) demonstration that apart from giving a life-like preservation, the nucleus itself could be followed up under the microscope during the cytological procedures like fixation, hydrolysis and staining.

The cells from the 7-day culture were centrifuged, washed well with distilled water and fixed in a freshly prepared mixture containing 9 ml. of Gram's iodine, 1 ml. of 40% formaldehyde and 2 drops of glacial acetic acid. After a stay of one hour in the fixative the cells were sedimented by centrifugation and smeared one cell thick on slides coated with Mayer's albumen. They were stored for one hour in 70% alcohol to remove the iodine and then washed in running water for 45 minutes. The slides were rinsed twice with distilled water and hydrolysed in N HCl at 60° C. for 8–10 minutes to remove the cytoplasmic basophilia (Sinoto and Yuasa, 1941; Royan, 1956 *a*).

Staining with Hæmatoxylin.—The hydrolysed cells were rinsed in distilled water and mordanted in 4% ferric ammonium sulphate for 16–24 hours. After a wash in running water for 30 minutes they were transferred to 0·5% well ripened hæmatoxylin for 48 hours. The smears were carefully differentiated in 2% iron alum, washed in running water for one hour, dehydrated

through ascending grades of alcohol, cleared in alcohol-xylol mixtures followed by pure xylol and mounted in canada balsam.

The structure of the zygote nucleus in iron-hæmatoxylin preparations (Photo 11) resembles that seen in the living zygotes. A well stained nuclear membrane delimits the nucleus from the cytoplasm. The formed structures visible inside the living nucleus are stained by hæmatoxylin. A comparison of Photos 7 and 11 may suggest that the stained nucleus is inferior to the living one in clarity. This is due to the technical difficulties involved in photographing nuclei having stained areas disposed at slightly differing foci.

Staining by the Feulgen technique.—For this purpose the hydrolysed smears rinsed well in distilled water were transferred to leuco-basic fuchsin and kept in the dark for 3 hours. They were differentiated in two changes of SO_2 water for 15 minutes each, lightly tinted with light green, dehydrated and mounted in canada balsam. Examination under ordinary illumination reveals a stained area (FSA, Photo 12) of variable configuration and size. The Feulgen positive area has little resemblance in structure to the body identified as the nucleus in the living cells (compare Photos 6, 7 and 8 with 12). If the nucleus was not visible in the living zygote, there is the likelihood of the Feulgen positive area being identified as the nucleus (Winge, 1935; Ganesan, 1956) in spite of differences between hæmatoxylin and Feulgen preparations. Lietz (1951) invites attention to the salient fact that the area stained by leuco-basic fuchsin forms only a portion of the nucleus. Heidenhain's hæmatoxylin is a regressive stain and one could stop differentiation at a stage to conform to the Feulgen picture (Ganesan, 1956). But the standard should be the living nucleus and not a Feulgen preparation.

Unfortunately the ordinary microscope does not reveal clearly the nuclear structure in Feulgen preparations. Examination under phase contrast becomes imperative. The structure of the nucleus is then clear. The Feulgen positive areas show varying dispositions inside the space bounded by the nuclear membrane (Photos 13 and 14).

DISCUSSION

A zygote is usually defined as the product of union of two gametes (Wilson, 1904). This term naturally embraces the individual originating from the product of fusion (Darlington, 1932). The buds arising from an yeast zygote constitute a population of individual cells. It would be desirable, therefore, to have a clear grasp of the connotation of the term zygote in yeasts.

Zygotes are reported to originate in yeasts in diverse ways (Phaff and Mrak, 1948). In some genera like *Schizosaccharomyces* the vegetative cells fuse in pairs to give rise to zygotes. Spore formation occurs in these zygotes (Royan, 1956 *d*). In the genus *Saccharomyces*, on the other hand, the vegetative cells give rise to asci with spores. Two spores when they fuse give rise to a spore zygote. In some cases the spores germinate directly and the resulting cells may fuse in pairs. The progeny of an isolated spore are reported to be capable of fusing with an ungerminated spore itself (Winge, 1935). It has been suggested that even the first two nuclei in a germinating spore may fuse to give rise to a diploid cell (Winge and Laustsen, 1940). During the formation of zygotes plasmogamy is presumed to be followed by karyogamy. In some cases, however, karyogamy is said to be delayed resulting in " dicaryotic " stages (Guilliermond, 1910; Renaud, 1938; Phaff and Mrak, 1948).

The zygotes illustrated (Photos 1–14) and described in this paper are formed by the fusion of pairs of spores. The nuclei of these zygotes have the structure suggested by Guilliermond. The existing reports on the nuclei of zygotes do not all relate to those formed by the fusion of spores. While Guilliermond's (1910) description is of dicaryotic stages in spore zygotes, Winge's (1935) observations relate to cell zygotes. It becomes relevant in this context to consider whether the divergences of opinion regarding the structure of the nucleus may be the result of differences in the mode of origin of the zygotes investigated by them. The similarity in the structure of the living nuclei of the zygote and bud illustrated in Photos 9 and 10 would imply that differences in the mode of origin may have no relation to the structure of the zygote nucleus.

The illustrations of the nuclei of living zygotes presented in this paper are from 7-day cultures. Naturally, these nuclei cannot be taken as representing the condition immediately on fusion of two spore nuclei. Since the nucleus of the zygote (Photo 9) retains its condition even after giving rise to the nucleus of the bud (Photo 10) it has to be presumed that the " fusion " nucleus has the same structure as the nucleus remaining in the zygote after giving rise to one or more buds. The similarity of structure of the nuclei of vegetative cells (Thyagarajan and Subramaniam, 1957) and zygotes (Photos 5, 6, 7 and 8) would reinforce such a conclusion.

Summary

1. The zygotes of *Saccharomyces carlsbergensis* retain the shape assumed at the time of their origin by the fusion of two spores even after they have produced a few buds.

2. The nucleus is visible in the zygotes on the 7th day after the introduction of spores into fresh wort. A nuclear membrane delimits the nucleus from the cytoplasm and encloses formed structures. The structure of the nucleus is identical in a zygote and its bud.

3. Iodine-formaldehyde-acetic gives a life-like preservation of cell structures. After removal of the cytoplasmic basophilia by acid hydrolysis, staining with hæmatoxylin gives pictures of nuclear details comparable to that of the living nucleus. The Feulgen stained area is only a portion of the resting nucleus.

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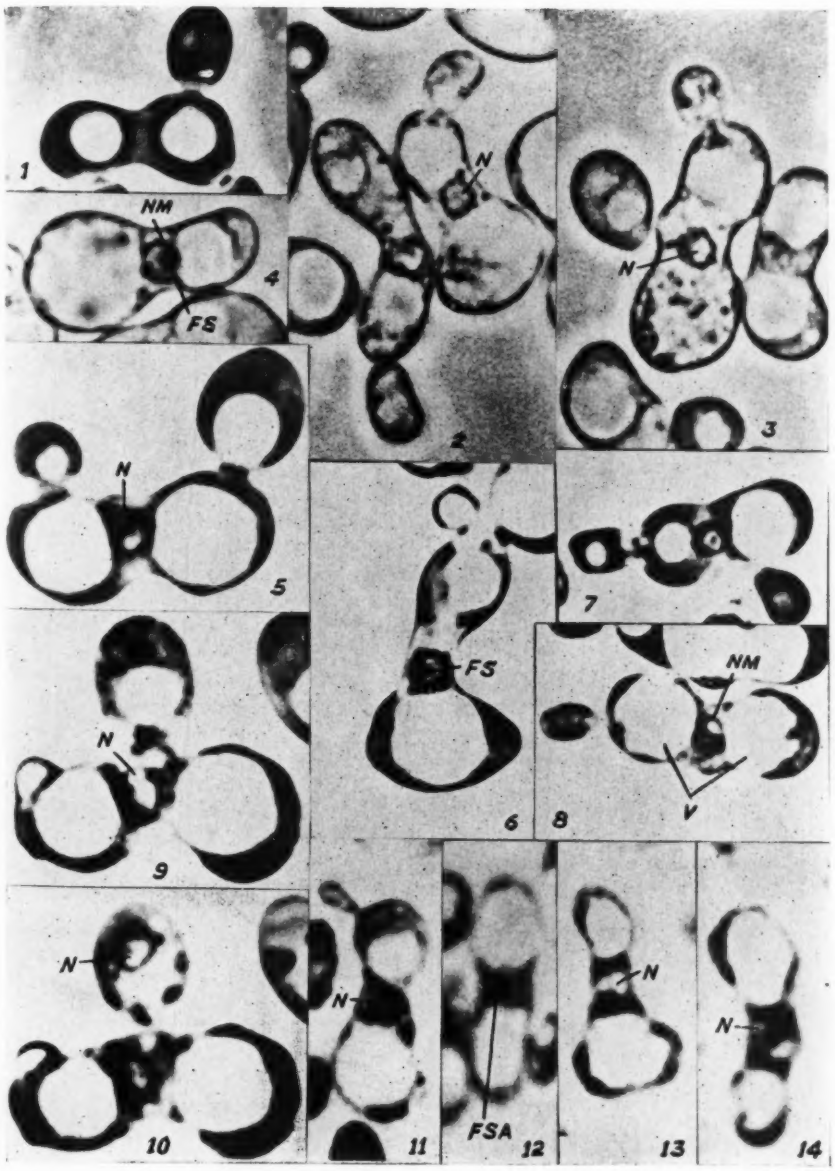
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Description of Photomicrographs

Photo 1. A zygote from a 3-day culture showing two vacuoles. The nucleus is not visible (Phase Contrast).

Photos 2, 3 & 4. Zygotes from 7-day culture. The nuclear membrane (NM) encloses formed structures inside the nucleus. A crescentic denser area attached to the inner surface of the nuclear membrane is visible in Photo 3, while a grain is present in Photo 4 (Ordinary Illumination).

Photo 5. A zygote with two attached buds of differing ages. The nuclear area is clear (Phase Contrast).

Photo 6. The nucleus has the appearance of a dense ring enclosing a distinct grain (Phase Contrast).

Photo 7. The nuclear periphery shows irregular thickening. Note the central grain (Phase Contrast).

Photo 8. A dense sickle-shaped mass is plastered on to the inner surface of the nuclear membrane (Phase Contrast).

Photos 9 & 10. Nuclei in a zygote and its bud (Phase Contrast).

Photo 11. The structure of the zygote nucleus after staining with hæmatoxylin (Ordinary Illumination).

Photo 12. The area stained by leuco-basic fuchsin in hydrolysed cells (Ordinary Illumination).

Photos 13 & 14. Under phase contrast the Feulgen positive areas show varying dispositions inside the space bounded by the nuclear membrane.

Magnification, ×4,200. N., Nucleus. NM., Nuclear Membrane. FS., Formed structures visible inside the nucleus. FSA., Feulgen stained area. V., Vacuole.

STUDIES ON THE ANATOMY OF THE SUGARCANE MOTH BORERS

II. External Anatomy of *Chilo tumidicostalis*, the Stem Borer of Sugarcane

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Introduction

THE sugarcane stem borer *Chilo* (*Argyria*) *tumidicostalis* Hampson (Plate XIII, Figs. 1–3) is the worst pest of its kind and has been reported to damage sugarcane crops in Assam, Bengal and certain parts of Bihar. Its earlier existence in the country is traceable since 1907 and 1911 from Assam and Bengal respectively as reported by Kapur (1950). Hampson (1919 *a*, 1919 *b*) described the specimens collected from Assam and Bengal as *Chilo gemininotalis* and *Argyria tumidicostalis* respectively. Later Fletcher and Ghosh (1919) and Ghosh (1921) also recorded it as *Argyria tumidicostalis*. Fletcher (1928) sank *Chilo gemininotalis* as a synonym of *Argyria tumidicostalis*. Kapur (1950) transferred this species *tumidicostalis* to genus *Chilo* and called it as *Chilo tumidicostalis*.

The study of anatomy of this moth borer has been very much neglected in comparison to other sugarcane moth borers and practically no work has been done on its morphology. Hampson (1919) and Kapur (1950) have given very brief descriptions of the adult moth and Puri (1957) has described in detail the morphology of the antennæ.

The present work therefore is an attempt to study in detail the external anatomy of the moth which may serve as an example of the structures usually associated with other sugarcane moth borers of the family *Pyralidæ*.

Material and Methods

The moths of *Chilo tumidicostalis* were reared from the field collected larvæ and pupæ. The larvæ were reared in the laboratory in cane pieces while the pupæ were placed over in petri dishes in which moist cotton was provided below the piece of a blotting-paper of equal size to cover the entire surface of the bottom of the dish, this being covered by a glass bell-jar.

The external study of the moths was carried on after soaking the specimens in 15% KOH solution for about 24 hours, thorough washing under a current of water for about eight hours, dehydrating in different grades of alcohol, clearing in carbo-turpentine, turpentine and then in cedar wood oil to mount it in the turpentine balsam in order to prepare the permanent slides of different parts as described by Puri (1954). The wings were also treated with two different wing solutions suggested by Puri (1956) to denud the wings completely and to get rid of the covering scales in order to study the clear view of the wing venation of the wings. The specimens and different structures were stained with eosin in 90% alcohol before clearing these in cedar wood oil.

THE HEAD

(Plate XIII, Figs. 4, 5, 6, 7 and Plate XIV, Fig. 1)

Head capsule.—It is comparatively a simple structure with a few sclerites. In facial view it appears to be an incomplete globe. The frons is a broad somewhat abrupt convex sclerite demarcated laterally by the ocular sutures extending from the antennal sockets ventrally to the sub-genal sutures. Its width is greatest at the sides just between the two eyes. It is of a widely conical shape provided with a distinct corneous point at the apex. The apex of the frons is abruptly convex and forms an apical snout-like protuberance over the base of the mouth parts. Slightly arched frontal suture between the opposite antennal sockets separates the frons from the vertex or the rest of the cranium. It is separated from the clypeus by the fronto-clypeal or epistomal suture. A pair of compound eyes is situated on the dorso-lateral walls of the cranium and just near these two eyes below the bases of the antennæ are situated two lateral ocelli on the dorsal part of the head. There are no traces of third ocellus which is absent. The foramen magnum is a small aperture with its arms diverging widely and anteriorly and is located almost in the centre of the posterior surface of the head. The area of the posterior surface immediately surrounding the foramen magnum is distinctly depressed dorso-laterally. The anterior surface gradually levels up while approaching the eyes and the vertex.

The epicranial suture is represented by slightly arched frontal suture described above. The occipital suture forms internally a bridge which probably strengthen the posterior part of the epicranial walls. On reaching near the vertex it becomes obliterated. Each of its arm starts from the posterior articulation of the vestigeal or ramanent mandibles of the two sides and traverses on the cranial wall, posterior to the respective gena of the side. This suture serves as the only line of demarcation between the lateral and occipital region of the head. The post-occipital suture surrounds the occipital foramen dorso-laterally. Internally this forms a post-occipital ridge upon which are attached the cervical muscles responsible for the movement of the head. The ends of this suture are located near the downward bent ends of the posterior arms of tentorium.

The sub-genal sutures separate the frons and the clypeus from the sub-genal region and are arched above the frontal pits, clearly discernible as vertically elongated depressions marking the invaginations that form the anterior tentorial arms. Each sub-genal suture runs laterally from the posterior tentorial pit to the anterior articulation of the vestigeal mandible of its side,

Each compound eye is externally surrounded by a distinct circular groove or the ocular suture which demarcates the frons laterally and forms an internal circumocular ridge. The post-genal regions are separated from the post-occipital regions by the post-genal sutures. The antennal sutures boarder the antennal sockets demarcating the narrow antennal sclerites.

Frons is a well marked median unpaired, separate sclerite prominently convex extending from the frontal and ocular sutures dorsally and laterally respectively to the epistomal suture ventrally. It has no actual boundaries laterally and dorsally due to the absence of the well marked frontal sutures and the atrophying epicranial suture. The epistomal suture separates it from the clypeus. There is a distinct inverted V-shaped corneous point at its apex, a character which is of great diagnostic value in separating this species from the other species of moth borers of the same sub-family *Crambinæ*.

The clypeus is a small transverse biconvex or a band-like sclerite attached to the labrum.

Vertex is the large convex area of the top of the head which is not limited by any special suture. The lateral ocelli are present on this area and the median one reported in certain other lepidopterous insects is absent. It lies between the frons and the occiput, boardered laterally by the dorsal portions of the two ocular sutures.

Each gena is represented by a small, narrow, elongated sclerite lying in between the posterior half of the ocular suture and the occipital suture. Dorsally it extends upto the vertex while ventrally it is demarcated by the pleurostomal suture (the part of the sub-genal suture above the mandible).

The compound eyes are sub-globular prominent and very large structures, situated laterad to the head capsule. Their greatest development is ventroposteriorly and they project beyond the ventral and posterior regions of the head capsule. The inner margins are curved slightly and are approximated ventrally to some extent. Narrow ocular sclerites separate the eyes from the frons and the clypeus. Dorsally these sclerites apparently terminate at the antennal sockets and ventrally they appear to merge with the sub-genal region of the head. The development of the eyes has almost completely obliterated the genæ. The two lateral ocelli are very prominent structures. The occipital area occupies the spaces between the occipital and the post-occipital sutures and the post-occiput or the post-occipital region is an incomplete ring of sclerite all-round the foramen magnum and serves as a buffer area between the foramen magnum and the occipital arch. The occipital

foramen occupies a large portion of the posterior region of the head. The posterior arms of the tentorium form a bridge across and above the foramen magnum. Post-genæ occupy the spaces between the post-occiput and the eyes and extend anteriorly to the ventral surface of the head where they merge with the sub-genal region. The post-genal suture separates these areas from the post-occiput.

Tentorium (Plate XIII, Figs. 4, 5, 6, Plate XIV, Fig. 1, Plate XV, Figs. 1 and 7).—It is well developed and braces the head internally. It consists of a pair of anterior tentorial arms which arise from the anterior tentorial pits. The two tentorial arms extend anteriorly to the sides of the clypeus and backward through the interior of the head capsule, to meet the posterior tentorial arms forming the tentorial bridge over and above the occipital foramen near the outer ends of the later. The posterior arms of the tentorium arise from the posterior tentorial pits (Fig. 1, Plate XV) and constitute a transverse bar or the tentorial bridge. Each posterior tentorial arm on leaving the posterior tentorial pit of its side bends slightly inward and posteriorly to form the tentorial bridge.

Antennæ.—The antennæ (Plate XIV, Figs. 2–11) are long slender darkish brown in colour and consist of 50–58 segments besides the scape and the pedicel, the higher number usually being found in case of males. These are situated very close to the middle of the facial wall of the cranium. The scape (Plate XIV, Figs. 4 and 8) tapers at its proximal end and is broad at the distal end where the ventral wall is deeply emarginated to provide space for the projected portion of the ventral wall of the pedicel to fit in it. There are two articular knobs in the form of internal projections of the distal region at its dorso-lateral angles which articulate with the pedicel. The proximal rim of the scape has a deep concavity and is completely placed within the antennal socket. The rim of the socket close to the eyes develops a knob-like projection or the antennefer which articulates with the concavity of the scape. This articulation is of the ' Socket and Ball ' type. The pedicel is somewhat bulbous, concave distally and projected proximally to fit into the concavity of the scape. The distal concavity of the pedicel receives the proximal convexity of the following annulus of the flagellum. The internal apodeme braces the pedicel internally. Each joint of the flagellum has the similar arrangement and has developed a convexity at its proximal end and a concavity on its distal end. The convexity of each joint fits into the concavity of the one preceding it. Normally there is a well marked difference between the structure and form of antennæ of the opposite sexes. The antennæ in the male and female are lamellate, pectinate and filiform respectively. It

has, however, been observed that where the segments of the female antennæ are somewhat similar to those of male, the joints in the female are comparatively narrow. The first a few segments of the flagellum are more or less cup-shaped, rounded and close. All other segments are provided with a tooth-like sense cone laterally because of which these joints become serrate. The distal segment of the flagellum bears a sense cone at its apex. In the male the segments of the flagellum are broadly rounded at the dorsal margin and are rather distinctly dilated laterally to appear somewhat triangular in cross-section. On each of these segments there is a sub-apical and sub-basal row of long cilia.

Mouth parts.—The labrum (Plate XIV, Fig. 1) consists of a narrow transverse sclerite, situated on the ventral side, attached to the posterior end of the clypeus. It is produced ventrad into a mesal and two lateral lobes. The two large and prominent side lobes bearing long bristles are the pilifers while the small mesal lobe constitutes the epipharynx in part. The vestigeal or rudimentary mandibles are represented by small triangular areas behind the pilifers. These may slightly be covered by the pilifers. There is no point of articulation with the head capsule and there are no definite sutures demarcating the mandibles from the sub-genæ. The feeble sclerotization of the mandibles shows that they can hardly be of any effective use and are merely of vestigeal nature.

The maxillæ are the largest and most important of the mouth parts. The sub-cylindrical stipes is curved mesally and is almost completely concealed beneath the projecting portion of the sub-gena. It articulates laterally with the head capsule and is thus situated ventro-posteriorly to the vestigeal mandibles. The proximal end of the stipes articulates with a small triangular-shaped chitinous structure of the cardo. The cardo articulates with the sclerotized portions of the labium. The two four-segmented maxillary palpi are somewhat palmate in shape and are greatly reduced in size. These are borne on the anterior portion of the stipes near the point of attachment to the galea. The entire surface of each maxillary palpus is covered with a large number of longitudinally fluted scales of varying length. These palpi are a pair of brushes on either side of the proboscis.

The distal end of each stipes bears the proboscis which constitutes the greater part of the maxillæ. The proboscis is composed of two lateral pieces, which are held together by interlocking grooves and ridges. The opposed walls are thickened and concave and enclose between them a canal through which the liquid food or drink is sucked up by the sucking pump into which opens the basal portion of the food canal. The sucking pump is well

developed and its floor is formed by the hypopharynx. The presence of lacinia at the base of the galea or proboscis supports the view of Pradhan and Aren (1941).

The structure of the labium is rather indefinite and the sclerotized portions are limited to two small areas around the base of each labial palpus, and to a short narrow median strip proximad of the base of the proboscis. The remainder probably is represented by the membranous portion of the ventral surface of the head. The two labial palpi consist three segments each. The basal segment of each palpus is well developed and is curved forward and upward in such a way that the remaining segments are applied closely to the anterior surface of the head. The second segment is the largest and stout but the third one is relatively small. On the distal segment of each labial palpus there is a ventral pit, near the apex, lined with short hairs and apparently represents a sensory organ. The entire surface of the labial palp is covered with long hairs.

THE THORAX

Cervix (Fig. 1, Plate XV) is the intersegmental region lying in between the head and the thorax. It is almost entirely membranous sclerotization being limited to a pair of lateral cervical sclerites (Plate XV, Figs. 1, 7). Each lateral cervical sclerite is composed of two pieces or arms arranged in such a way so as to form a V-shaped composite lateral cervical sclerite. The two arms of each sclerite are hinged together at a point which forms the apex of the word V which articulates with the anterior margin of the prothoracic episternum and is prolonged beyond the point of articulation as a short thick free stem. The distal ends of the anterior or dorsal arms of each sclerite (Fig. 1) is produced into a peg-like projection to fit into the concavities of the occipital condyles of their respective sides thus affording an articulation between the head and the neck at the points on which the former can move freely. The posterior or the ventral arms of these sclerites articulate with the prothoracic episternum laterally and are united by a narrow bridge.

The prothorax (Plate XV, Figs. 1, 5, 6 and 7).—A good deal of confusion exists even today with regard to the form and structure of the reduced prothoracic plates in *Lepidoptera*. Comstock (1948) described the pronotum produced on each side, into a flat lobe, constricted at the base as a stalked plate or the patagium. Imms (1951) described patagia as lobe-like structures frequently carried by the prothorax at its sides which may also assume the form of collar-like lobes. Snodgrass (1935) does not make any mention of these structures at all. Madden (1944) suggested the patagia, pronotal plates and the parapatagia to be the parts of the lepidopterous prothorax. The

present author is of the opinion that the pronotal plates (Plate XV, Fig. 6) are curved and rounded to form lobe-like structures as described by Imms (1951) and the patagia (Figs. 2, 5 and 6) are the lateral outgrowths of these plates supported dorsally by the pronotal plates. These patagia assume the form of collar-like, longer errectile expansions in these insects, there being no parapatagia as described by Madden in case of Sphingiadæ.

The prothorax (Plate XV, Fig. 6) according to the present observations is composed of three pronotal plates which are arranged in such a way that mesally these give rise to a structure Y in shape in between the two lateral lobes formed by these plates and their lateral outgrowths or the patagia. The free stem or the posterior prolongation of the word Y is produced into a vertical peg-like projection articulating posteriorly with a bowl-shaped concavity of the mesothoracic prescutum. The ventral margins of the pronotal plates are partially fused with the episternal sclerites.

The episternum (Plate XV, Fig. 7) is a convex sclerite partially fused with the ventral margins of the pronotum. The pleural suture is quite obvious and forms a prominent pleural ridge which gives rise to a long slender pleural arm on each side to join the sternal furca near the sternal pits of its respective side. The episternum is rather fused with the anterior portion of the sternum by a faint and narrow precoxale and the postcoxale attains its separate entity and connects the epimeron with the sternum behind the coxal cavity.

The presternum is well defined and runs horizontally as an internal apodeme. It is partially fused with the episternum and is also connected with the basisternum and sternellum by the so-called strap-like spinasternum. At its sides it also receives the post-coxale and the inturned ventral edge of the trochantin. The basisternum is reduced to a very narrow region representing the edges of a deep median fold of the transverse internal ridge or the sternacosta. It is separated from the sternellum by a well marked sternacostal suture which extends right upto the sternal pits in the form of a broad U. The spinasternum is represented by a vertical median strap-like process which forks into two lateral arms posteriorly. The trochantin (Plate XV, Fig. 7) is a large plate bounded by the pleural arm, its own inturned ventral edge and the post-coxale.

Mesothorax.—It is the largest and most prominent of the three segments of the thorax. The prescutum (Plate XV, Figs. 2, 4, 5 and 6) is a small sclerite lying anterior to the scutum, directed ventrad and is almost invisible from the dorsal aspect. A narrow bowl-shaped depression occurs near its anterior margin. Laterally its margins are produced into two lateral prealares which form the prealare bridge. The prescutal ridge formed by the prescutal suture

sets off the prescutum from the scutum. The scutum (Plate XV, Fig. 2) is the largest of the thoracic tergites. Its anterior margin is cleft and the lateral margins caudad to the prealares provide for the notal tegular incision. A little beyond this point each lateral margin is produced or flattened into a suralare, which serves as an anterior pivotal point for the wing. The median suture which generally forms the median carina in the scutal region in other moths including the sugarcane moth borers is almost completely obliterated.

*Notal sub-tegular wing processes, the so-called tegulæ are well developed and each is carried on a special tegular plate supported by means of a tegular arm. Its antero lateral margin is produced below and backward which passes beneath the base of the forewing. It appears to be somewhat triangular independent sclerite when seen from the dorsal surface. The entire surface of the tegulæ is clothed with scales and small bristles or setæ. The postero-lateral margins of the scutum consist of flat extensions on either side each containing two lobe-like outgrowths. The anterior lobe or adanale is long and slender and serves as the posterior articulatory point for the forewing, while the posterior lobe is shorter and stouter and represents the postadanale. Between the suralare and the adanale, the lateral margin of the scutum is extended into a forward projecting adnotale which functions also as an articulatory point for the wing. The axillary cords of the basal wing membranes seem to arise just posterior to the postadanales at the sides of the posterior marginal fold of the scutellum. The scutellum is more or less a rhombus or heart-shaped tergite lying posterior to the scutum. Its anterior two sides are bounded by the reversed V-shaped (Λ) endotergal ridge of the scutoscutellar suture which lies with its apex directed forward, dividing the notum into an anterior scutum and a posterior scutellum. The two posterior sides of the scutellum are marked by the intersegmental fold of antecostal ridge between the scutellum and the metathoracic scutum. A post-transscutellar suture forms an internal ridge which cuts through the posterior part of the mesothoracic scutellum bracing it internally. Posterior to and below the mesoscutellum, behind the internal ridge lies the post-scutellum which bears the second phragma. This plate represents the second postnotum with which is incorporated the acrotergite of the meta thorax. The intersegmental fold between the post-scutellum and the metathoracic scutum forms a huge post-phragma which is developed

* The author has explained in detail the position and status of the Notal sub-tegular wing processes in Part IV (to be submitted) and is of the opinion that tegulæ are different from these notal sub-tegular wing processes which seem to be connected with the Tnoracic notum rather than with the wings.

to such an extent that the dorsal organs and the alimentary tract are forced into the ventral region of the body cavity in order to pass beneath.

The pleuron is divided by a pleural suture into an episternum and an epimeron. A transverse anepisternal suture divides the episternum into a dorsal anepisternum and a ventral katepisternum which continues as a narrow precoxale and fuses with the furcasternum. The lobe-like pre-episternum fuses ventrally with the basisternum and is separated from the katepisternum by the pre-episternal suture. A fold in the dorsal margin of the anepisternum may represent the basal portion of the basealare but the basealare proper is a small triangular plate lying dorsad of the fold.

The epimeron is almost V-shaped and a distinct suture cuts off the anterior portion into somewhat a sub-circular region which Shepard (1930) called the pre-epimeron. The anterior margin of the pre-epimeron overlies the pleural suture and its upper portion lies in a cavity parallel with the suture and extends forward into the anepisternum. The narrow antero-dorsal portion of the epimeron overlies this cavity and is produced dorsally into a pleural wing process. The epimeron tapers to a sharp point immediately caudad of the meron and does not form a post-coxale.

The sub-alare lies in the membrane above the epimeron and is invaginated mesally to support the important wing muscles. A small narrow plate, which apparently represents the second sub-alare, is located directly behind the sub-alare. The mesothoracic spiracle (Plate XV, Fig. 7) has migrated forward and lies in the pleural membrane of the prothorax caudad of the patagia.

The basisternum is a broad wedge-shaped sclerite which is inrolled to form a midventral suture resembling an internal keel-shaped ridge. The furcasternum is a small triangular deeply infolded plate, and the median invagination forms a pair of broad furcal arms which are directed slightly caudad. The ends of these arms fuse with the posterior portions of the epimera to form a brace across the caudal end of the meso thoracic cavity. There is no apparent connection between the furcal arms and the pleural ridge which is a very unusual condition. However, the structure of the endoskeleton is so complicated by additional invaginations and infoldings that this point cannot be determined definitely.

Metathorax.—The scutum (Plate XV, Fig. 2) consists of two lateral lobe-like sclerites somewhat triangular in shape with arms almost across. The anterior margins are produced slightly to form the suralares and the flattened posterior margins are produced into two adanales.

The scutellum is a transverse triangular plate with its apex directed forward and lies across the base of the scutum. The lateral margins of the scutellum are produced into membranous axillary cords, which extend to the anal region of the hind wings. The post-scutellum lies immediately caudad of the scutellum and is represented by a deep invagination forming a large internal phragma for attachment of the muscles. The membranous portion of the first abdominal tergite is attached to the posterior margin of the post-scutellum. Two vertical ridges or convergent ridges formed by the so called convergent sutures are very prominent throughout the entire posterior part of the notum and cut through the metascutum and the metascutellum forming a median area in each case. Posteriorly these convergent ridges are bent and continuous with a transverse curved ridge looking like a single convergent ridge. The presence of these convergent ridges braces the entire structure of the thorax and is quite unusual and undescribed by the workers in the past. Anteriorly these are continuous with the antecostal ridge which runs along the sides of the mesothorax anteriorly. The scutellum is cut apart into three parts by these convergent arms and one may be mistaken in tracing out its boundaries. The median or the biggest piece lies in the centre. The convergent ridges (Plate XV, Fig. 2) arise antero-laterally and extend posteriorly usually converging towards the median line of the back.

The metapleuron is divided by a vertical pleural suture into an anterior episternum and a posterior epimeron. The episternum fuses ventrally with the basisternum and on its dorsal margin, there is a weakly sclerotized subcircular pad which Shepard (1930) called the anterior basealare or basealare pad.

The metathoracic spiracle lies directly beneath the basealare pad and the small quadrate basealare is located immediately above it while the small vertical pleural wing process lies caudad of the basealare. The sub-alare lies in the membrane above a deep emargination in the dorsal margin of the epimeron. A latero-ventral extension of the post-scutellum fuses with the posterior region of the epimeron to form a postalare bridge the upper portion of which becomes deeply infolded and merges with the invagination of the post-scutellum.

The basisternum is reduced to a very narrow precoxale in front of the coxal cavity. The furcasternum is reduced correspondingly and the apophyses arising from it branch dorsally into an anterior and a posterior pair of furcal arms. The anterior arms are short and stout while the posterior arms are much longer and fuse with the ventral margin of the epimeron to form a narrow post-coxale, attached to the basal abdominal sternite. There

is no apparent connection between the furcal arms and the pleural ridge which corresponds with the condition existing in the mesothorax.

Legs (Plate XVI, Figs. 1, 2 and 3).—The coxa of the prothoracic leg is an elongate, cylindrical segment which is broadest near the base and tapers towards the distal end. It articulates proximally with the trochantin but the coxa of the meso and metathoracic legs are immovably united with the pleura. The latter are divided by a basicostal suture into an anterior eucoxa and a posterior meron which are produced distally into trochantifers articulating freely with the condyles of the trochanter. A triangular plate at the base of the coxa of the meso and metathoracic legs, lying between the katepisternum and the meron, is separated from the eucoxa by the basicostal suture. Snodgrass (1909) calls this plate the trochantin but Shepard (1930) calls it epicoxal piece.

The trochanters of all the legs are sub-globose and of about equal size. The only point of articulation is between the condyles and the trochantifers. The distal end is immovably attached to the femur in each case.

The posterior margin of the femur of the prothoracic leg is broadly rounded proximally but tapers towards the distal end, thus providing a space for the accommodation of the epiphyses when the femur and the tibia are folded together. The femora are clothed with hairs but do not bear spines, spurs or setæ.

The tibia of the prothoracic leg is rather short and stout and is provided proximally on the inner surface with a pair of lamellate spurs or epiphyses which rest in a long cavity in the posterior margin when the legs are folded. The tibia of the mesothoracic leg is about twice the length of the prothoracic tibia. It is slender but widens towards the distal end which is armed with a pair of stout spurs. The outer spur is about twice the length of the inner. The metathoracic tibia is more than twice the length of the prothoracic tibia and is armed with two pairs of spurs, one at the distal end and the other at about 2/3rd the length of the tibia. The longer spur of each pair is on the outer side.

The tarsus of each leg is composed of five segments. The basitarsus is the longest in all the legs. The segments become progressively shorter and the shortest is the distitarsus. There are four to five rod-like spines of medium size on the anterior margin of the basitarsus of the prothoracic legs. Each tarsus is clothed with hairs and bristles.

A pretarsus (Plate XV, Fig. 4) is present at the tip of the distitarsus of each leg. It is composed of two large curved ungues which articulate with

the dorsal margin of the distitarsus by means of small hook-shaped unguifers. The membranous pulvilli arise from the membrane at the base of the claw and proximad of the bases of the claws, there is a broad sclerite with a median hair-like elongation at the distal margin. The basal portion of this sclerite apparently represents the planta while the distal elongation is probably the empodium. The arolium has been reduced to a small membranous area above the empodium and bears a tiny, sclerotised orbicula. The base of the planta is separated slightly from the sclerotized unguitractor which is partially withdrawn into the distitarsus.

Wings (Plate XVI, Figs. 5 and 6).—The forewings (Fig. 5) are somewhat elongate and triangular in shape. The apical angles are acute. The outer margins are crenulate and somewhat shorter than the inner margins which are slightly sinuate. The forewings are 13–14 mm. long with an average of 13·5 mm. The maximum width of the wings is about 5–6 mm. The wing venation which is interpreted according to the Comstock-Needham system, is described below:—

SC is well developed, separate and unbranched, R 1 arises from near the middle of the cell while R 2 and the stalk of R 3 and R 4 originate from before the angle of the cell. The stalk is longer than either R 3 or R 4. R 5 originates from the apex of the cell. M 1 is discocellular originating from the angle of the cell. Its point of origin is near to R 5 than M 2. M 2 and M 3 arise from the cell, near the posterior angle or in close proximity but are not fused. The cross vein PV is very fine and slightly bent inwardly, closes the cell distally. The two branches of CU 1 are present and CU 2 is completely obliterated. CU 1 *a* arises from before the lower angle of the cell and CU 1 *b* from the middle of the cell. The first anal seems to have atrophied, A 2 is present and A 3 is also absent. This observation of the present author is contrary to that of Gupta (1940) who described A 1 to be present in case of *Chilotræa infuscatellus* (*Argyria sticticraspis*), and is supported by a direct microphotograph in which A 1 appears to be a rudimentary vein near the base of the wing and the entire rudimentary vein appears as a rudimentary fold (Plate XVI, Fig. 5). R4, R5, M 1, M 2, M 3, CU 1 *a*, CU 1 *b* are equidistant with each other on the margin of the wing while A2 is situated distantly from CU1 *b*.

Hind wing (Plate IV, Fig. 6).—SC *plus* R 1 and RS arise free but anastomose closely beyond the cell to diverge again into SC *plus* R 1 and RS. M 1 originates from RS before its fusion with SC *plus* R 1. The fine cross vein PTV makes an angle PTV which is almost nearly a right angle. The area of the cell is reduced due to the drawing in of the cross vein towards the base of the wing. M 2 and M 3 arise in a common stalk from the lower angle of

the cell and diverge later into M 2 and M 3. CU 1 originates from the base of the wing and diverges into CU1 *a* and CU1 *b*. It appears that CU1 acts as a common stalk for M 2, M 3, CU1 *a* and CU1 *b*. A 1, A 2 and A 3 are all present. A frenulum projects forward from the humeral angle of the hind wing. In the male the frenulum is composed of a single stout spine directed beneath the forewing. In case of female the frenulum consists of usually three stout spines or three spines and a long fine spine-like bristle situated near the base of the second stout spine (Plate XVII, Figs. 1 and 2). This indicates the trend of reduction in the number of spines constituting the frenulum in these insects. The tips of these spines are received in a cluster of hairs (Plate XVII, Fig. 2) collectively known as the retinaculum, which is situated on the ventral surface of the forewing. The function of the frenulum is to co-ordinate in the movement of the wing in flight.

The wings are attached to the body by articular membranes which contain several axillary sclerites. The humeral plate at the anterior margin of the base of the forewing articulates with the base of the costal vein. The first axillary lies caudad of the humeral plate and the anterior arm articulates with the lateral margin of the suralare and the head of the subcosta. The posterior portion articulates with the adnotale while the antero-lateral portion articulates with the second axillary which in turn articulates with the base of the radial vein and the pleural wing processes. The flexor muscles of the wings are inserted on the triangular third axillary which lies caudad of the second. The proximal median plate lies distad of the second and the third axillaries and the distal median plate lies slightly distad of the first axillary. Caudad of the proximal median plate and the third axillary there is a basal knob or basoplica. Similar articular axillary sclerites occur in the hind wing with the exception of humeral plate which is modified into frenulum and the distal median plate.

The Abdomen and Genitalia

Structure of the abdomen (Plate XIII, Figs. 2 and 3).—It is elongate, conical in shape and is composed of ten segments. The last two to three segments, however, are modified to form the genitalia and are retracted within the last visible segment. There are only eight apparent segments in the male and seven in the female. The sclerotized portions of the segments consist of a tergite and a sternite. These are separated by a membranous pleural region bearing the seven abdominal spiracles. The segments are connected by conjunctival membranes and each tergite and sternite normally overlaps the following tergite and sternite. A narrow acrotergite is demarked by a faint antecostal suture in the tergites of the unmodified segments except the first.

This suture forms an internal antecosta upon which the principal muscles are attached.

The first abdominal tergite is greatly reduced and supports a long narrow plate on its lateral margin. This plate extends forward and fuses with the edges of a fold in the posterior margin of the metathoracic post-scutellum. A deep groove occurs in this plate, which represents the tergo-pleural groove. A small pleural sclerite lies below the plate (Plate XIII, Fig. 3). The anterior portion of the second tergite is extended into the pleural region and probably represents the second abdominal pleurite, although it is not demarcated from the tergite by a suture. None of the remaining segments seem to have sclerotized areas in the pleural region.

The first abdominal sternite fuses with the second to form wide plate beneath both the first and the second tergites. There is a deep invagination near the upper anterior margin of this plate which gives rise to a hook-shaped process upon which certain muscles are attached. The orifice of a scent organ lies in the pleural membrane above the dorsal margin of the second sternite (Plate XIII, Fig. 3) in the male and a groove extends backwards from this opening to a point above the fourth sternite. The surface of the orifice is covered with a tuft of long hairs.

Male genitalia (Plate XVII, Figs. 5, 6 and 7).—The male genitalia are composed of highly modified ninth and the tenth abdominal segments. The posterior part of the eighth abdominal segment does form a protractile base for the copulatory apparatus. The male genitalia of some of the sugarcane moth borers in particular, belonging to the sub-family *Crambinæ*, have been studied and described by Gupta (1940) and Kapur (1950). These authors have used the terminology suggested by Busck and Heeinrich (1921). The present author has endeavoured, however, to use the terminology which is in common use to-day and has been used and described by Mehta (1933) and Snodgrass (1935) while describing these structures in Lepidoptera.

The tegumen apparently represents the ninth tergite. It is strongly arched dorsally and tapers slightly towards its posterior extremity. The tergum fuses with the ninth sternite, the coxosternal part of which is rounded and drawn out posteriorly to be known as Vinculum. It is extended forward in a deep inflection known as the Saccus, the membranous ventral wall of which is adnate with the sternal surface.

Lying within the vinculum is a quadrate slightly bent plate with a pair of longitudinal arms. This is known as anellus. Its arms, which are curved upward near the base, are directed forward distally and lie along the sides of

the ædeagus. Each arm is provided with a pair of sharp conical teeth at the external margin. One of these teeth is situated in the middle while the other is present at 2/3rd the length of the arms and is more prominent (Plate XVII, Fig. 6). The chitinized shield or plate borne on the ventral surface of the anellus has been named as Juxta by some Lepidopterists. The ædeagus is a long sclerotized tube which serves as a protective armature and guide for the penis. It is divided for the distal 3/4th of its length, the lower part slightly shorter and flattened into a bilobed structure which is widely channelled to accommodate the upper part which is rather club-shaped. The ædeagus is slightly narrowed in the middle and pointed at the apex. It is weakly chitinized dorsally and provided with a dense cluster of hairs in the sub-apical region.

The paired harpes are quadrate and articulate with the coxopodital or posterior region of the vinculum. The ribs or costa of these structures are almost straight. The harpes are heavily sclerotized and slightly dentate at the posterior apex. The inner surface of each is slightly concave and covered with long hairs. The ventral region of the harpes, produced into a curved inner process, has been called as sacculus by some Lepidopterists. The distal portion of this process consists of a ventral prong. The inner dorsal angles of harpes are connected by a transtilla with deeply invaginated ends which form articulatory points for the harpes.

The anal armature belongs to the tenth segment and is composed of two parts, *viz.*, the uncus and the gnathos, which are partially fused at the base. The uncus probably represents the tenth tergite and is a single ventrally inclined hook projecting over the anus. It is provided with many short setæ. The gnathos consists of a pair of arms and probably represents the modified tenth sternite. The uncus and the gnathos (Plate XVII, Fig. 7) together look like a beak-like structure in the lateral view. Ventrally these appear to be somewhat triangular in shape and the plate bearing these two is nearly as long as wide.

Female genitalia (Plate XVIII, Figs. 1, 2, 3 and 4).—The eversible female genitalia are normally retracted within the seventh somite and are still further concealed by a thick clothing of long hairs.

The ostium bursæ or the genital opening of the bursa-copulatrix lies on the ventral surface of the eighth abdominal segment on a heavily chitinized oval plate. Within this oval plate (Fig. 3) the genital opening proper is guarded laterally by two strongly chitinized sword-shaped arms with their apices directed forward. These arms are supported posteriorly on a thick transverse chitinized plate bounding the genital opening posteriorly. These

arms probably function as a guide for tracing out the genital opening for the penis of the male during the process of copulation. The genital opening is in the form of a folded pocket in the upper half, in between the two arms, and is sunk in the median oval plate. The rim of the external oval plate is provided with a heavily sclerotized band. The posterior plate supporting the two sword-shaped arms, guarding the genital opening, joins the rim of this oval plate posteriorly. The anterior half of the ductus bursæ is heavily sclerotized at the sides and the posterior half is only slightly chitinized. Bursa copulatrix is sausage-like, three to four times as long as wide and the star-shaped signum reported to be present on the bursa copulatrix of some other sugarcane moth borers is absent.

The ovipositor (Plate XVIII, Fig. 4) consists of a chitinous ring resembling a broad sole of a modern shoe. It is profusely studded with setæ and bristles. The chitinous ring is supported by chitinous stylets. The anus and the opening of the ovipositor lie in between the two longitudinal lobes, constituting the entire ovipositor, which appear to be almost completely fused. The oviporus lies ventrad of the anus and represents the external opening of the egg duct. It forms also a posterior opening of the genitalia. Two short fingerlike or band-like sclerotized processes lie directly behind and below the oviporous. These probably function as auxillary guides in placing the eggs. The ovipositor represents the united ninth and the tenth somites as stated by Snodgrass (1933).

SUMMARY

1. A detailed anatomical description of the adult moth of the sugarcane stem borer, *Chilo tumidicostalis* is presented as an example of the structure of the entire family *Pyralidæ*.

2. The head capsule is a simple structure and the shape and the structure of the frons is typical for the species. It is provided with a corneous point at the apex, a diagnostic feature in the head. The mouth parts are greatly reduced, maxillæ being the most highly developed of the mouth parts. The maxillary palpi are four-segmented and are palmate in shape. The labial palpi are three-segmented and are provided with a sensory pit at the distal segment near the apex. The labium is reduced to two rounded sclerotized areas around the bases of the labial palpi.

3. The cervix is almost entirely membranous, sclerotization being limited to two lateral V-shaped sclerites which are dorsally covered by the patagia.

4. The prothorax is greatly reduced and the three pronotal plates dorsally support the patagia. The parapatagia are absent. The mesothorax is greatly enlarged while the metathorax is somewhat smaller with prominent lateral lobes representing the metathoracic scutum. A reduced post-scutellum is also present in addition to the scutellum which is triangular in shape.

5. The structure of the legs has been described in detail. All the legs are provided distally with a well developed pretarsus.

6. The wing venation has been studied and described in detail. In the forewing A 1 is absent, A 2 is present and A 3 is again absent.

Tegulæ are well developed and are situated as kno-blike structures at the anterior root of the wing base. These are supported by the tegular arms which are long and extend upto the posterior notal wing process. The antero-lateral margin of the notal sub-tegular wing process is bent below and extend posteriorly to cover the bases of the forewings. The frenulum in the female is composed of three stout spines and a thin long bristle while in the male it is represented by a single stout spine.

7. The abdomen is spindle-shaped and consists of ten segments, although there appear to be only eight segments in the male and seven in the female.

8. The male genitalia are composed of the modified ninth and tenth abdominal segments and their different parts have been described in detail.

9. The female genitalia are composed of eighth, ninth and the tenth abdominal segments and are as important in identification of this species as are the male genitalia. The structure of the different parts has been described in detail.

Acknowledgments

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EXPLANATION OF PLATES

PLATE XIII

FIG. 1. Fresh male and female moths.

FIG. 2. Female moth (whole mount).

FIG. 3. Male moth (Whole mount without head).

FIG. 4. Head capsule.

FIG. 5. Head and mouth parts (Ventral view).

FIG. 6. Head capsule, mouth parts and patagia (dorsal view).

FIG. 7. Head capsule, mouth parts (Lateral view).

PLATE VIV

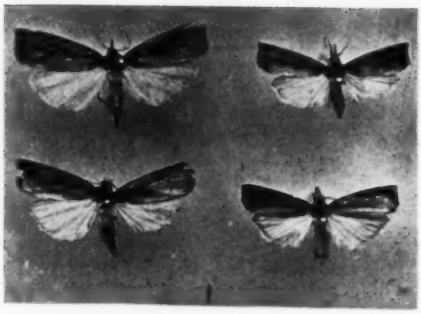
FIG. 1. Head and mouth parts: A, Dorsal; B, Ventral; C, Anterior tentorial pits, lacinia and pilifers; D, Labial palpus; E, Maxillary palpus; F, Pilifer.

FIG. 2. Male antenna.

FIG. 3. Female antenna.

FIGS. 4, 5, 6 & 7. Parts of the male antenna.

FIGS. 8, 9, 10 & 11. Parts of the Female antenna.



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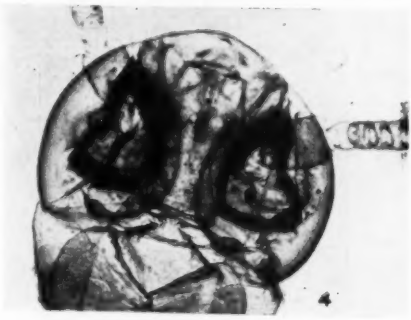
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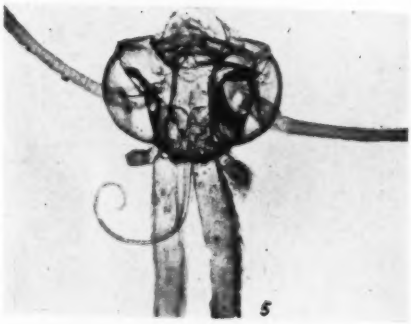
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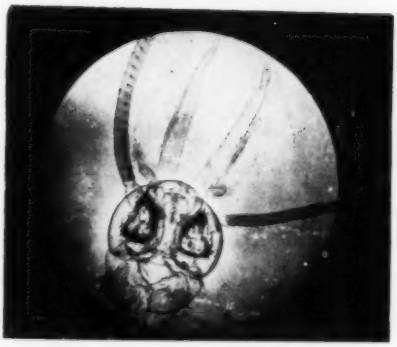

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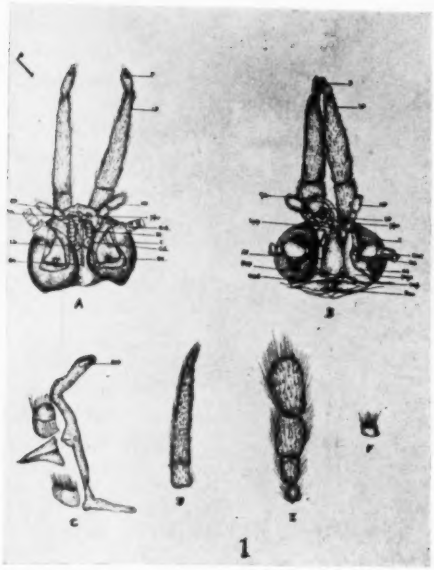
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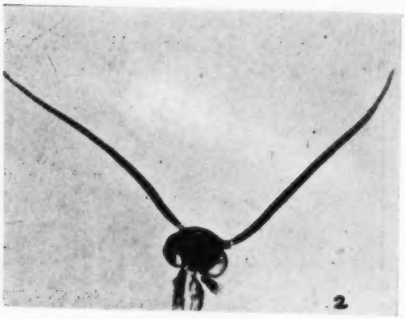
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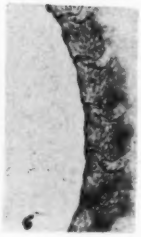
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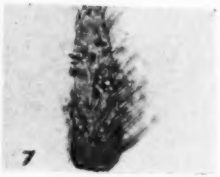
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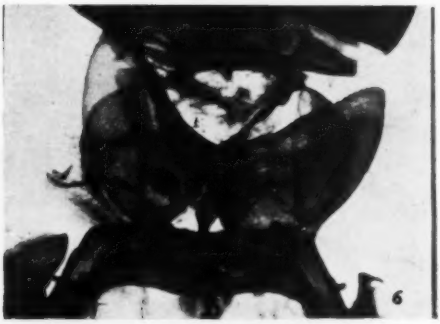
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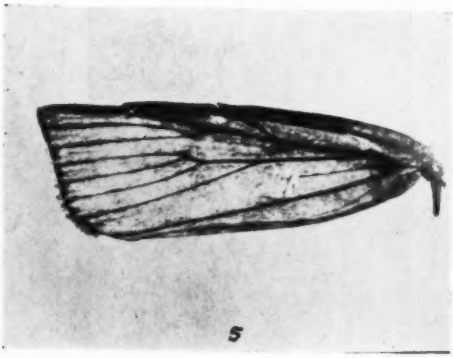
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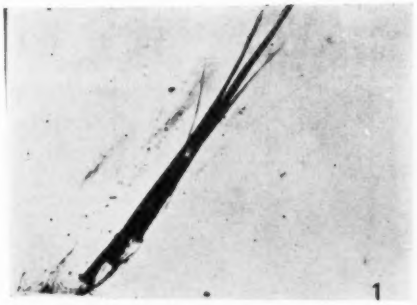
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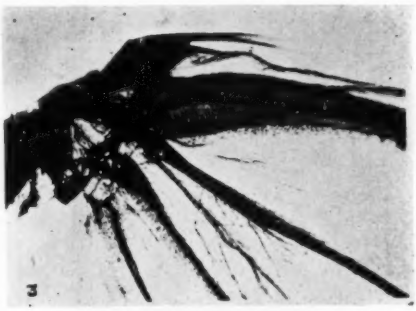
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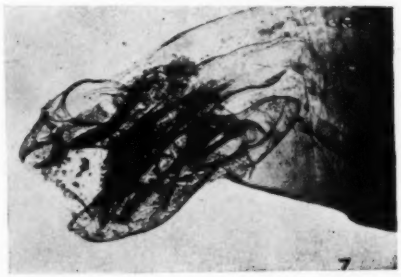
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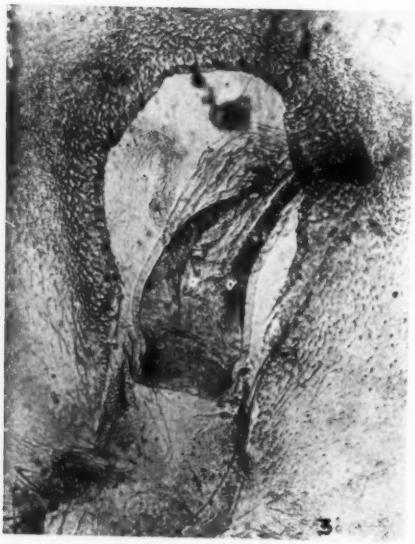
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PLATE XV

FIG. 1. Cervix, occipital foramen and partly tentorium.
FIG. 2. Thorax (Dorsal view).
FIG. 3. Thorax (Lateral view).
FIG. 4. Thorax (Ventral view).
FIG. 5. Head, neck and thorax together.
FIG. 6. Prothorax and patagia with a part of mesothorax.
FIG. 7. Prothorax (Ventral view) with cervix and the posterior part of the head.

PLATE XVI

FIG. 1. Fore leg.
FIG. 2. Mesothoracic leg.
FIG. 3. Hind leg.
FIG. 4. Pretarsus.
FIG. 5. Forewing.
FIG. 6. Hind wing.
FIG. 7. Notal sub-tegular wing process.

PLATE XVII

FIG. 1. Enlarged view of the frenulum in female.
FIG. 2. The frenulum and the retinaculum.
FIG. 3. Humeral plate modified into frenulum.
FIG. 4. Axillary sclerites of the forewing.
FIGS. 5, 6 & 7. Male genitalia.

PLATE XVIII

FIG 1. Female abdomen and genitalia.
FIG 2. Same (enlarged view).
FIG 3. Position of the genital opening.
FIG 4. Ovipositor.

KEY TO THE LETTERING

ant., Antenna; *as.*, Antennal suture; *AT.*, Anterior tentorial arm; *ATP.*, Anterior tentorial pit; *Cd.*, Cardo; *E.*, Compound eye; *EP.*, Epipharynx; *ga.*, Galea; *hyp.*, Hypopharynx; *Lb.*, Labium; *lm.*, labrum; *Lp.*, Labial palp; *MP.*, Maxillary palp; *OC.*, Ocellus; *Ocd.*, Occipital condyle; *Ocf.*, Occipital foramen; *Os.*, Ocular suture; *P.*, Sensory pit; *Pfr.*, Pilifer; *pge.*, Post-genæ; *Por.*, Post-occipital ridge; *PTP.*, Posterior tentorial pit; *St.*, Stipes.

FLOWER STRUCTURE AND ANTHESIS IN THE GENUS *ALYSICARPUS*

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(Communicated by Prof. L. S. S. Kumar, F.A.SC.)

The improvement of forage legumes by breeding methods, has assumed considerable importance due to the fact that our grass lands are devoid of suitable species capable of growing in association with the grass flora. Recently, Solomon and Shama Rao (1955) have published data collected on 'Pollination studies in the genus *Alysicarpus*' which contains several species fulfilling the above requirements. It is proposed in this paper to give further details regarding flower structure and anthesis in the above genus, as this is of considerable interest to research workers.

Flower Structure

The inflorescence in the species *A. pubescens*, *A. longifolius*, *A. buplurifolius*, *A. vaginalis* and *A. rugosus* var. *styracifolius* and *heyneanus*—Types A, B and C, is of racemose type, composed of several paired flowers which in themselves are complete units of reproduction. The racemes are mostly terminal but also axillary growing continuously during the flowering period. The florets develop and open in an assending order from the base to the top of the rachis. The flowers are sessile in *A. pubescens*, sub-sessile in *A. rugosus* var. *styracifolius* and Pedicelled in others. They are zygomorphic having a large posterior petal—the 'Standard' or 'Vexillum,' two laterals—the 'Wings' or 'Alæ 'and two lower petals united to form a 'Keel' or 'Carina'. The calyx has five sepals, the odd sepal being anterior, while the posterior sepals are united. The 'Standard' is crimson lake in *A. pubescens* and in others faint chrome yellow in colour. The wings are rose to pink while the keel is generally pink only at its apex.

The andræcium consists typically of ten stamens which are diadelphous with nine united into a tube and a single free stamen lying along its upper side. The staminal column encloses the style and redivides into its nine constituent stamens near the top while the free stamen is attached to its base with a long filament. The filament of the alternate anthers are of equal size, arranged in two whorls surrounding the stigma. The staminal tube and the filaments of the anthers are white, while the anther lobes slightly yellowish.

The gynæcium consists of a long incurved style and a terminal capitate stigma. The style protrudes beyond the anther lobes in the flowers of the species *A. rugosus* var. *heyneanus*—Types A, B and C, *A. pubescens* and *A. longifolius*; while it is level with the surface of the anthers in the case of *A. rugosus* var. *styracifolius*, *A. buplurifolius* and *A. vaginalis*.

Process of Anthesis

The most striking feature of the flower is the tripping or exploding mechanism. In this respect these flowers resemble those of ' Alfalfa ' described by Gray (1925) and Kirk and White (1932–33).

When anthesis begins, a slight cleavage can be observed at the apex of the bud, as the apex of the sepals will now be free. Gradually the united posterior sepals covering the ' Standard ' begin to separate and a few minutes later, the others, *viz.*, the two laterals and one ventral enclosing the ' wings ' and the ' keel ', respectively. The posterior sepals will still be in contact with the back of the ' standard,' while the laterals and the anterior ones will now be free from the ' wings ' and ' keel ', respectively.

At this stage, the corolla will be slightly exerted from the calyx. When the corolla starts opening, all the sepals simultaneously fall wide apart. Now the well stretched ' standard ' and the ' wings ' closely adhering to the keel are clearly visible. This can more or less be considered as the half opened flower stage, since the staminal column and the style will have been still enclosed within the keel. Generally five to thirty minutes are taken to reach this stage, depending upon the temperature and humidity of the atmosphere. Higher the temperature quicker the process of opening and *vice versa*. The united posterior sepals at the back of the ' standard ' will now be free except at the apex which will be in contact with the mid portion of the ventral surface of the ' standard '; thus aiding as a stake for the latter to stretch firmly so as to make it more conspicuous for the insects. The lateral and the anterior sepals will still be free of contact with the ' wings ' and ' keel ', respectively. The ' keel ' is folded longitudinally all along its margin, and the margin of the fold is upwards. The fold will be intact at its apex where it encloses completely the stamens and the stigma, while it forms a slight groove at its back. On the keel are two processes in the nature of evaginations which lead to the slight groove in front of the staminal column and prevent it from springing forward. The processes of the wings seem to interlock with the evaginations of the keel and thus further augment the resisting force. This half opened flower condition persists till insects visit these flowers.

When the flowers are tripped by insects or by hand, the staminal column with the style will be released from the keel. The keel which was hitherto

erect and folded, becomes boat-shaped and slightly folds back so that its back rests on the anterior sepal which was hitherto free. The wings adhering very slightly at the base of the keel will now lie on either side of it and are slightly twisted. This is the fully opened condition of the flower. When the flower fully develops, due to its weight the pedicel bends downward, slightly. About ten minutes after the visit of an insect or any other agency which brings about tripping, the well stretched 'standard' involutes, *i.e.*, rolls from both margins to the middle of the upper surface and thus assumes the form of a trumpet. The apex of the staminal tube along with the style will now be enclosed within this trumpet-shaped standard. Thirty minutes after the release of the staminal tube from the keel, the flower starts drooping and the 'standard' completely encloses the staminal tube. The 'standard' comes in contact with the keel, the latter at this stage being almost enclosed on either side by the wings. The posterior and anterior sepals are brought nearer simultaneously with the movement of the 'standard' and the 'keel'. An hour after tripping, all the flowers will have crumpled, lying in one mass, while the lateral sepals encloses the posterior sepal on either side. These three sepals completely enclose the crumpled petals and a few minutes later all these are enclosed partially by the anterior sepal of the keel; thus resembling the original condition of the bud.

The whole process of blooming to closing of the various floral organs in the case of the flowers visited by insects or tripped artificially is completed within two hours. In the case of the untripped flowers, the half-opened condition of the flower persists uptill 4 to 5 P.M. Later, the 'standard' begins to curl inwards and encloses the staminal tube. The rest of the process will be similar to that of the tripped ones. In either case, the fresh condition of the flower persists only for a few hours.

DAILY DISTRIBUTION OF FLOWERING

In order to know the number of flowers blooming per day, thirty plants were observed in each species and types at intervals of an hour from 8 A.M. to 12 Noon. Every time after counting, the flowers were plucked so that there should not be any confusion while counting latter. The means with thier standard error for the thirty observations made in the species and types are presented in Table I.

It will be seen that between 8 and 9 A.M., very few flowers bloomed in all the species and types observed. In fact, blooming was observed to commence after 8–30 A.M. It will be noticed that from 9 A.M. onwards, blooming was considerable upto 10 A.M. Between 8 and 9 A.M., the temperature rose from 72·7 to 76·2° F., and the humidity fell from 89 to 74 per cent.;

TABLE I

TABLE I

Means with their standard errors for the number of flowers bloomed at hourly intervals from 8 a.m. to 12 Noon per plant per day in the species and types observed

	Alysicarpus rugosus				*A. pubescens*	*A. longifolius*	*A. buplurifolius*	*A. vaginalis*
	var. *styracifolius*	var. *heyneanus*						
		Type (A)	Type (B)	Type (C)				
Total number of flowers bloomed between 8 A.M. to 12 Noon Temp. Humidity Max. 85·3° F. Max. 91% Min. 69·5° F. Min. 46%	169·7 ±4·3266	360·9 ±18·6349	258·9 ±3·0513	590·0 ±11·0114	80·5 ±1·4628	76·93 ±1·798	102·96 ±2·1557	90·8 ±0·6517
Number of flowers bloomed between 8 and 9 A.M. Temp. Humidity 72·7–76·2° F. 89–74%	19·6 ±0·813 (11·8%)	16·23 ±0·6167 (4·4%)	29·12 ±0·961 (11·2%)	90·68 ±1·0191 (15·1%)	11·17 ±0·7916 (13·7%)	1·3 ±0·013 (1·5%)	3·46 ±0·079 (3·3%)	13·2 ±0·761 (14·5%)
Number of flowers bloomed between 9 and 10 A.M. Temp. Humidity 76·2–79·3° F. 74–64%	119·6 ±1·8312 (70·0%)	242·3 ±2·731 (67·1%)	126·7 ±1·1312 (49·0%)	384·0 ±1·991 (64·4%)	58·26 ±0·8821 (72·5%)	36·92 ±0·5771 (48·0%)	60·2 ±0·8336 (58·4%)	45·0 ±0·5971 (49·6%)
Number of flowers bloomed between 10 and 11 A.M. Temp. Humidity 79·3–80·5° F. 64–57%	30·23 ±0·6317 (17·6%)	100·6 ±1·798 (28·0%)	100·82 ±1·6561 (39·0%)	120·91 ±2·413 (20·3%)	10·0 ±0·3123 (12·5%)	32·61 ±0·9162 (42·8%)	35·31 ±0·8381 (34·2%)	30·23 ±0·9012 (33·3%)
Number of flowers bloomed between 11 A.M. and 12 Noon Temp. Humidity 80·5–83·4° F. 57–48%	1·11 ±0·0361 (0·6%)	1·71 ±0·0169 (0·5%)	2·5 ±0·0216 (0·8%)	1·0 ±0·0171 (0·2%)	1·21 ±0·0317 (1·3%)	6·13 ±0·0837 (7·7%)	4·32 ±0·0567 (4·1%)	2·37 ±0·0673 (2·6%)

whereas, between 9 A.M. and 10 A.M., the temperature rose from 76·2 to 79·3° F., while the humidity decreased by 10 per cent., *i.e.*, from 74 per cent. to 64 per cent.

Further, it is seen that the difference in temperature between 104 and 11 A.M., was only 1·2° F., *i.e.*, it rose from 79·3 to 80·5° F., and the humidity fell from 64 to 57 per cent. The number of flowers blooming is less at this period as compared to the period between 9 and 10 A.M., except in the case of *A. longifolius* where there is not much variation in the number of flowers blooming during these two periods. From 11 A.M. onwards with a further rise in temperature and fall in humidity, the blooming was practically negligible in all the species and types observed.

Thus the optimum period for the flowers to bloom is between 9 and 11 A.M., and the optimum temperature and humidity required being 76·2 to 80·5° F. and 74 to 57 per cent., respectively, as almost 90 per cent. of the flowers opened during this period.

The data for the different species and types was reduced to percentages and these figures were used for drawing Fig. 1.

DURATION OF FLOWERING

From each species and type, ten plants were selected at random and in each plant ten pairs of racemes of nearly equal size and same stage of development were marked. All the pods and the wilted flowers on these racemes were cut off, a day prior to commencing observation. These inflorescenses were labelled and the plants were visited from 8 A.M. onwards, daily. A record of the observations made from middle of August to middle of November 1952 is presented in Table II.

From Table II it is seen that of the species studied, the earliest to flower was *A. vaginalis*. Later, *A. buplurifolius*, *A. rugosus* var. *styracifolius* and *A. rugosus* var. *heyneanus*—Type A, started flowering; and the last to flower were *A. pubescens*, *A. longifolius* and *A. rugosus* var. *heyneanus*—Types B and C. *A. rugosus* var. *styracifolius* and Type A of var. *heyneanus* first started flowering, Type B nearly a week later; and Type C, after about a fortnight. Thus starting of flowering differ in the three types; Type A being early, Type B intermediate and Type C late, respectively.

It will also be seen that flowering occurred in two flushes in the species *A. longifolius*, *A. buplurifolius* and *A. vaginalis*, the duration of the second flush of flowering being considerably less as compared to the first.

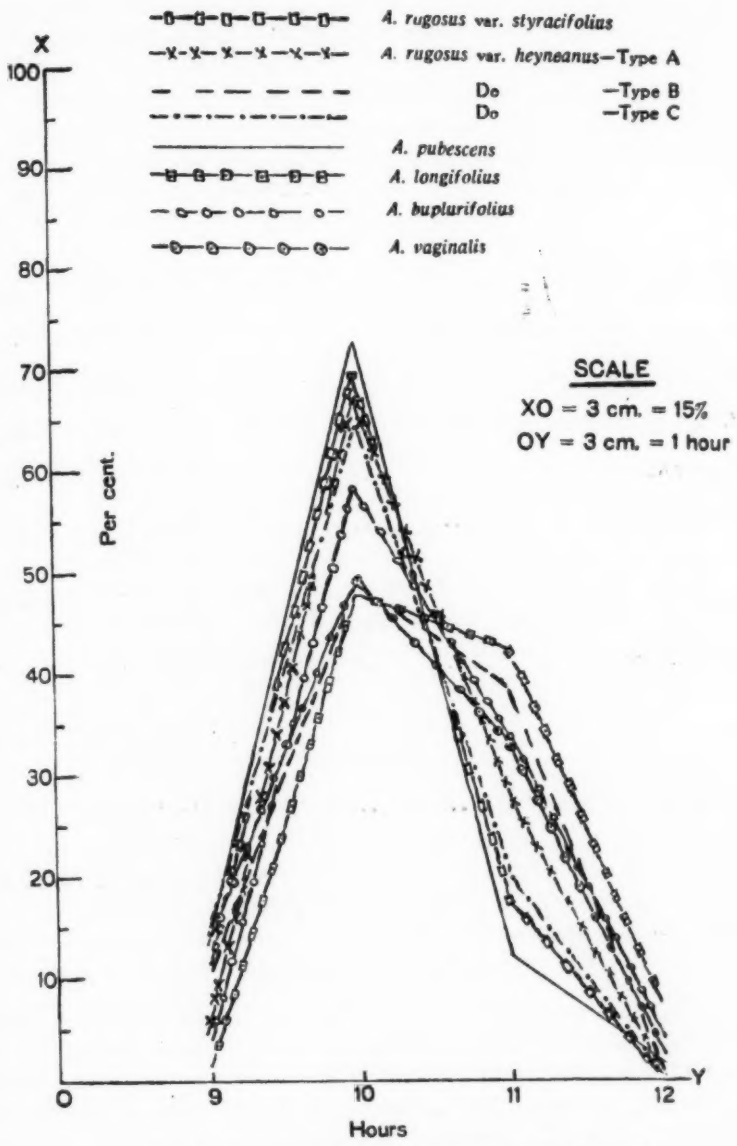

FIG. 1. Percentage of Flowers bloomed at hourly intervals.

TABLE II

Dates of commencement and closing of flowering in some species and types in the genus Alysicarpus

Date of Sowing: 1-6-1952

Sl. No.	Species	Date of commencement	Date of closing	Duration of flowering
1	*A. rugosus* var. *styracifolius*	15- 8-1952	2-10-1952	48 days
2	*A. rugosus* var. *heyneanus*—Type A ..	11- 8-1952	30- 9-1952	50 days
3	*A. rugosus* var. *heyneanus*—Type B ..	24- 8-1952	16-10-1952	53 days
4	*A. rugosus* var. *heyneanus*—Type C ..	3- 9-1952	7-11-1952	65 days
5	*A. pubescens*	24- 8-1952	23-10-1952	60 days
6	*A. longifolius*	28- 8-1952 (I flush)	19-10-1952 (I flush)	52 days
		24-10-1952 (II flush)	30-10-1952 (II flush)	6 days
7	*A. buplurifolius*	15- 8-1952 (I flush)	22- 9-1952 (I flush)	38 days
		2-10-1952 (II flush)	17-10-1952 (II flush)	15 days
8	*A. vaginalis*	8- 8-1952 (I flush)	19- 9-1952 (I flush)	42 days
		2-10-1952 (II flush)	20-10-1952 (II flush)	18 days

DEHISCENCE OF ANTHERS

The anthers are apiculate, rounded at the base and bilobed with a longitudinal septum in the middle. Dehiscence takes place longitudinally on either side of this central axis. It commences when the 'standard' begins to separate from the keel in the bud and is completed when the flowers are in the half-open stage, *i.e.*, between 9 and 10 A.M.; they are now ready for tripping. When these flowers are tripped either by insects or by hand, the staminal tube releases from the keel and the pollen dust is tossed up in the

form of a fine spray due to the upward jerk of the anthers. In the species where the stigmatic surface is level with the anthers, some pollen grains were seen being deposited on the stigmatic surface, thus bringing about self-fertilization. In the species where the style protrudes well beyond the anther lobes, the pollen dust were seen being deposited on the back of the incurved stigma and very rarely on the stigmatic surface. Thus, self-pollination is hindered in these species.

Receptivity of Stigma

In the buds which are to open on the next day, the style is tender, pale green and only partially developed. On the next morning, those in the late bud stage will have their styles grown to their normal length. In the species *A. rugosus* var. *styracifolius*, *A. buplurifolius* and *A. vaginalis* the surface of the stigma is in level with the surface of the anthers, whereas, in *A. rugosus* var. *heyneanus*—Types A, B and C, *A. pubescens* and *A. longifolius*, the style protrudes 1 mm. beyond the anther lobes.

The receptive condition of the stigma can easily be detected by the slimy deposit oozing out of its surface. The surface of the stigma will then be moist and shining and any pollen falling on it sticks to its surface. In *A. pubescens*, *A. rugosus* var. *styracifolius*, *A. buplurifolius* and *A. vaginalis*, the slimy exudation on the stigmatic surface can very well be observed from 9–30 A.M. onwards. In *A. rugosus* var. *heyneanus*—Types A, B and C, the stigma becomes receptive from 9–40 A.M. onwards; whereas in *A. longifolius* from 9–45 A.M. onwards.

Summary

There are many species of forage value in the genus *Alysicarpus*.

The optimum period for the flowers to bloom is between 9 and 11 A.M., the optimum temperature and humidity required being 76·2 to 80·5° F. and 74 to 57 per cent., respectively. Flowering starts in the middle of August and continues for about two months. In *A. longifolius*, *A. buplurifolius* and *A. vaginalis*, flowering takes place in two flushes; the second flush being of considerably smaller duration than the first.

Dehiscence of anthers commences when anthesis begin, and is completed when the flower is ready for tripping, *i.e.*, between 9 and 10 A.M.

The stigma is receptive in all species studied, between 10 and 11 A.M. The time required from blooming to closing of the several parts, is about two hours.

In the species *A. rugosus* var. *styracifolius*, *A. buplurifolius*, and *A. vaginalis*, the surface of the stigma is in level with the surface of the anthers; whereas, in *A. rugosus* var. *heyneanus*—Types A, B and C, *A. pubescens* and *A. longifolius*, the style protrudes 1 mm. beyond the anther lobes. Self-pollination generally occurs in the species where the stigmatic surface is in level with the surface of the anthers, but the same is usually prevented in the species where the style protrudes well beyond the anther lobes.

ACKNOWLEDGEMENT

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ON THE INFLUENCE OF SULPHA DRUGS IN SUGARCANE

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I. Introduction

The utility of sulpha drugs as bacteriastatic agents in animal physiology is well known. Many workers from time to time have tested the effect of these drugs on different plants and plant processes as well. However, the results obtained were quite diverse and variable. Thus, Sulphonamides were found to inhibit germination of oats and wheat (Bustinza and Ahvaro, 1945; Bhardwaj & Rao, 1954) as also of other higher plants (Euler, 1948). On the other hand, improved germination was found in *Hibiscus esculentus* (Kumar & Srivastava, 1953). Increased seedling mortality has also been reported by Bhardwaj and Rao (1954). The inhibiting action of Sulphonamides on root growth has been observed by a host of workers covering a wide range of higher plants (Grace, 1938; Woods, 1940; Macht and Kehoe, 1943; Euler, 1948; Andus and Quastel, 1948; Kayser and Benson, 1948; Bhardwaj and Rao 1954; Bhardwaj, 1954). Stimulating effect on root growth has also been recorded in *Hibiscus esculentus* (Kumar and Srivastava, 1953) and sugarcane (Rao, Prasad and Khanna, 1956). Both inhibition (Andus and Quastel, 1948) and stimulation (Kumar and Srivastava, 1953) were regarded as distinct from Sulphonamide bacteriastasis. Inhibited growth of seedlings has been observed in barley, rye, *Lolium perenne*, *Phleum pratense*, *Lepidium sativum*, *Vicia faba* (Euler, 1948), flax (Moore, 1953) and wheat (Bhardwaj and Rao, 1954). On the other hand, Rao, Prasad and Khanna (1956) have reported stimulated shoot growth in sugarcane. Bhardwaj and Rao (1954) observed that tillering was induced early and earing delayed by 30 days with shrivelling of grains in wheat. Rao *et al.* (1956), working on sugarcane, found general improvement in stalk weight, leaf number, fresh weight of roots, stem and whole plant, dry weights of sheath and root, moisture content of leaf, sheath and whole plant as also in yield and juice quality resulting from sulphanilamide treatment. Garo (1947) showed in *Hyapolum senatum*, depression of transpiration due to complete closing down of the stomata when treated with Sulphamides. Spraying of wheat seedlings with the drug solution led to chlorosis and even necrosis; however, the plants recovered in about 7 days (Hotson, 1953). Woods (1950) stated that the primary action

of Sulphamides is competitive inhibition of an enzyme reaction (or reactions) in which PABA is involved; the chief function of PABA is its requirement in the synthesis of folic acid and the inhibition of this reaction is the principle point of attack of the drugs in the cell. Moore (1953) interpreted this results as suggesting that in the flax seedlings Sulphanilamide interferes with the conversion of pamino benzoic acid to Pteroyl-glutamic acid. Sivaramakrishnan and Sharma (1953) have also studied the effect of sulpha drugs on the pamino benzoic acid metabolism with lebelled C^{14} in *Phaseolus radiatus*. Audus and Quastel (1948) thought that the effect in general varied with the drug and its concentration.

The above results showed the varied responses obtained in different crops, leading to the conclusion that the influence of these sulpha drugs varied largely with species and as such it was thought relevant to analyse its influence on the growth of sugarcane.

II. MATERIALS AND METHODS

One budded sets of variety B.O. 17 were planted in February in pots filled with the local calcareous loamy soil. A basal dose of nitrogen (as ammonium sulphate) and Phosphorus (as single super) at 60 and 75 lb./acre respectively was applied at the time of planting. Five sulpha drugs, *viz.*, Sulphanilamide, Sulphadiazine, Sulphaguanidine, Sulphamerazine and Sulphathiazol were under study. Two doses (184 and 368 p.p.m.) of each drug were applied at the third month stage to the soil; another dose of 122 p.p.m. was applied at the 4th month stage to another set of pots. In all 10 cultures for treatment were maintained. Irrigations and hoeings whenever necessary were given to maintain optimum conditions of growth.

Periodical observations on growth and other attributes were recorded along with yield and juice quality at harvest.

III. EXPERIMENTAL FINDINGS

Within a week of application of the drugs, leaves started yellowing—degree of chlorosis being directly proportional to the dose, with specific variation among the different drugs. Thus in Sulphanilamide, Sulphaguanidine and Sulphadiazine, all the leaves became entirely chlorotic while in Sulphathiazol it was confined only to the intercostal regions (Fig. 1). In Sulphamerazine there was just a suggestion of chlorosis. At the lowest dose of 122 p.p.m., chlorosis appeared only with Sulphanilamide. But by the end of the 3rd month, leaf colouration was restored.

All the drugs in all concentrations tried retarded root growth, the retardation being directly proportional to the concentration of the drug (Fig. 2).

Shoot growth in turn was also retarded and the effect varied with the drug and its concentration. In case of Sulphanilamide (184 and 368 p.p.m.), Sulphaguanidine (368 p.p.m.) and Sulphadiazine (368 p.p.m.) plants stopped growing altogether and died off within a month, least inhibition being under Sulphamerazine (184 p.p.m.) and Sulphathiazol (184 p.p.m.). With 122 p.p.m. dose, growth was retarded only with Sulphanilamide; with others it was as good as that of control. With the passage of time, the treated plants picked up the rate of growth and by the end of the 5th month, cultures under Sulphamerazine (184 p.p.m.) were on par with control (Table I).

TABLE I

Height of mother shoot in inches

Drugs	Dose	Age in days				
		90	113	140	173	205
Sulphaguanidine ..	Control	5·47	13·80	19·81	30·60	40·40
	1·5 gm.	5·50	8·40	16·11	27·11	36·40
	3·0 gm.	5·50	6·50	..	..	..
Sulphadiazine ..	Control	6·60	16·34	21·5	33·24	40·2
	1·5 gm.	5·87	8·7	12·67	25·17	32·97
	3·0 gm.	5·47	6·50	..	..	..
Sulphathiazol ..	Control	6·8	12·0	19·5	29·17	36·2
	1·5 gm.	7·6	13·4	18·0	23·4	30·57
	3·0 gm.	7·77	12·57	17·24	19·0	25·4
Sulphamerazine ..	Control	9·51	18·50	26·50	36·80	45·5
	1·5 gm.	8·91	14·40	25·50	37·8	45·67
	3·0 gm.	7·60	8·50	15·20	25·2	32·67

Within a fortnight after the application of the drug, the tissues at the lower nodes in all the treatments, excepting Sulphamerazine (both doses) and Sulphathiazol (184 p.p.m.), became meristematic and produced an undifferentiated mass of tissues, with a few distinct bud forms. Many of these germinated later on, to form healthy tillers (Fig. 3). The quantity of adventitious tissue formed, again varied with the drug and its concentration. In decreasing order, the drugs can be arranged as follows: Sulphanilamide, Sulphadiazine and Sulphaguanidine, Sulphathiazol and Sulphamerazine. With the lower dose of 122 p.p.m., no adventitious tissue was formed except with Sulphanilamide where, in a few plants, mild symptoms of such

structures were visible. However, in all the plants treated with Sulphanilamide, bud size and enlarged and many of them had sprouted along with the adjoining root band to form water-suckers. The number of green leaves also varied with the drug and was inversely proportional to its concentration (Table II).

TABLE II

Number of green leaves (Mother shoot)

Drugs	Dose	Age in days				
		90	113	140	173	205
Sulphaguanidine ..	Control	0·67	0·67	1·34	1·34	2·67
	1·5 gm.	1·00	1·00	1·67	1·00	2·00
	3·0 gm.	1·00	1·00	..	..	..
Sulphadiazine ..	Control	0·34	0·34	2·00	1·00	1·34
	1·5 gm.	0·67	0·67	1·00	1·34	1·34
	3·0 gm.	0·67	0·67	..	..	..
Sulphathiazol ..	Control	0·34	0·34	0·34	0·34	0·34
	1·5 gm.	1·00	1·00	1·00	1·00	1·00
	3·0 gm.	0·67	0·67	0·67	1·00	1·00
Sulphamerazine ..	Control	0·67	0·67	0·67	0·67	0·67
	1·5 gm.	1·00	1·00	1·00	0·07	1·00
	3·0 gm.	0·67	0·67	0·67	0·34	0·34

The drugs showed a general tendency to improve the number of healthy tillers per clump (Table III)—the best result having been obtained with Sulphamerazine and Sulphathiazol (184 p.p.m.). However, an indication of higher doses being toxic was clearly discernible. Here the cluster of tillers formed by the sprouting of buds in the adventitious mass as excluded, most of which ultimately died off, due probably to competition among themselves.

The weight of canes per clump decreased steadily with increase in concentration of the drug—the only treatment having equalled the value of control being 184 p.p.m. dose of Sulphamerazine (Table IV).

So far as the various quality attributes like extraction %, Brix, sucrose, purity and glucose were concerned, no systematic trends were discernible (Table V).

TABLE III

Total number of tillers per clump

Drugs	Treatments	Age in days				
		90	113	140	173	205
Sulphaguanidine ..	Control	0·67	0·67	1·34	1·34	2·67
	1·5 gm.	1·00	1·00	1·67	1·00	2·00
	3·0 gm.	1·00	1·00	..	..	..
Sulphadiazine ..	Control	0·34	0·34	2·00	1·00	1·34
	1·5 gm.	0·67	0·67	1·00	1·34	1·34
	3·0 gm.	0·67	0·67	..	..	..
Sulphathiazol ..	Control	0·34	0·34	0·34	0·34	0·34
	1·5 gm.	1·00	1·00	1·00	1·00	1·00
	3·0 gm.	0·67	0·67	0·67	0·67	0·67
Sulphamerazine ..	Control	0·67	0·67	0·67	0·67	0·67
	1·5 gm.	1·00	1·00	1·00	0·67	1·00
	3·0 gm.	0·67	0·67	0·67	0·34	0·34

TABLE IV

Yield in Oz./Clump

Sl. No.	Drugs	Control	Dose		
			1·0 gm.	1·5 gm.	3·0 gm.
1	Sulphanilamide	9·7	4·3	..	..
2	Sulphadiazine	7·7	6·7	6·5	3·0
3	Sulphaguanidine ..	9·3	8·3	5·7	4·7
4	Sulphamerazine ..	7·3	6·7	7·3	5·0
5	Sulphathiazol	7·3	6·7	4·0	3·3

IV. DISCUSSION

Application of various sulpha drugs to cane brought about interesting effects. The first symptom to appear was chlorosis. This may be possibly

TABLE V

Juice characteristics

Drugs	Dose	Extraction %	Glucose	Brix	Sucrose	Purity
Sulphaguanidine	Contro.	55·12	0·888	20·6	14·317	68·08
	1·0 gm.	50·00	0·783	18·8	15·536	78·08
	1·5 gm.	..	0·816	20·7	15·558	75·83
	3·0 gm.	..	2·203	22·5	14·220	63·78
Sulphanilamide	Control	55·12	0·725	19·6	15·631	79·85
	1·0 gm.	50·00	0·788	20·2	16·150	81·16
Sulphadiazine..	Control	56·71	1·672	20·0	16·557	82·87
	1·0 gm.	59·97	0·659	19·6	15·822	78·27
	1·5 gm.	62·50	0·636	21·75	16·499	76·71
	3·0 gm.	50·00	1·813	19·5	14·979	76·82
Sulphamerazine	Control	53·40	0·152	23·3	19·03	78·95
	1·0 gm.	50·00	0·202	20·5	16·130	78·45
	1·5 gm.	50·00	0·269	21·9	15·223	71·29
	3·0 gm.	50·00	..	21·2	13·962	66·26
Sulphathiazol..	Control	52·94	..	21·3	17·271	83·84
	1·0 gm.	50·00	..	21·3	15·227	72·38
	1·5 gm.	66·75	..	21·0	18·137	64·31
	3·0 gm.	51·38	..	21·0	14·775	70·62

due to a retarded absorption of essential elements, like magnesium and iron for the formation of chlorophyll, resulting in chlorosis and consequent inhibited carbon assimilation, ultimately leading to retarded root and shoot growth. Since the normal metabolic state of leaf is disturbed, a retarded production of auxins may also be visualised. Snow (1932) in *Picum sativum* found that the larger leaves have also an inhibitory effect on lateral buds and its power increases with the age of the seedling. Thimann and Skoog (1934 *a*, 1934 *b*) observed that in the leaves also growth substances are produced but in lesser amounts than in the bud. Looking at the extent of foliage developed in sugarcane, if it is presumed that production of growth substances is considerable, the retardation in the production rate of these auxins might explain to a certain extent the sprouting of lateral buds and the production of adventitious tissues on the nodes. However, the explanation does not seem to be plausible, since retarded root and shoot growth, though reported by many workers as stated earlier, has not been accompanied with chlorosis

except in wheat (Hotson, 1953) and even there when the plants were sprayed ærially.

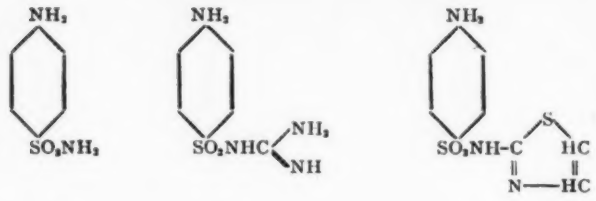
Alternatively, a disturbance in the enzymatic complex may also be put forward. These sulpha drugs are known to form metal complexes and if they do so with the prosthetic groups of enzymes it might result in chlorosis and might also explain other phenomena observed, *viz.*, retarded root and shoot growth, sprouting of lateral buds and production of adventitious tissues on the nodes, in as much as these changes are the basis of the physiological effects caused by auxins (Overbeek, 1952). The observations recorded thus seem clearly to bring auxins into the picture. However, Audus and Quastel (1948) thought that root growth inhibition caused by these drugs is a phenomenon quite distinct from that produced by plant growth substances. It seems that synthesis of auxins in the apical bud and leaves is either retarded or is rendered inactive as soon as it is produced, resulting in the reduction of apical dominance and leading to sprouting of lateral buds and production of an uncontrolled mass of adventitious tissues. Went (1951) observed that deficient auxin supply in the first place prevents normal elongation of the main shoot, and secondly that there is an increased lateral bud development, leading to witches brooms.

Skoog and Tsui (1951) reported that increased KH_2PO_4 increased bud formation and quoted R. W. Howell as definitely ascribing this effect to H_2PO_4 radical. It would be interesting to see whether these sulpha drugs stimulate H_2PO_4 absorption.

It thus seems that a host of factors must be responsible for the profound effect of these sulpha drugs on sugarcane.

The sprouting of lateral buds and production of adventitious buds point to the possibility of increasing tillering by working out the proper drug and its concentration, time of application, etc.; for, if the lower buds are induced to sprout early in the life-cycle, they can avail of the advantage of the grand period of growth and may grow into mature canes, thereby holding out promise for increased yields.

As to the drugs themselves, an interesting correlation has been observed between the constituents of the side chain and the effects produced. Taking Sulphanilamide (I) and its two derivatives, *i.e.*, Sulphaguanidine (II) and Sulphathiazol (III).



I II III

into consideration it seems clearly to stand out that as the length of side chain increases the extent of disturbances produced decreases. Finally in Sulphathiazol a 5-membered ring also comes to be incorporated and this might explain the mild effect of this drug for, as a rule, 5-membered rings do not confer activity; however, the fusion of a 6-membered ring with a 5-membered ring is supposed to confer activity (Thimann, 1951).

Such being the profound effects of these sulpha drugs, further experiments in micro plots have been laid out and their results would form the subject-matter of a separate communication in due course.

V. SUMMARY

Influence of five sulpha drugs, *viz.*, Sulphanilamide, Sulphadiazine, Sulphaguanidine, Sulphamerazine, and Sulphathiazol has been studied under pot culture conditions.

Variations in growth exposition as influenced by these have been described with a critical discussion in the context of the latest available literature on the mechanism of these drugs.

Further possible lines of work have been put forward.

VI. ACKNOWLEDGEMENT

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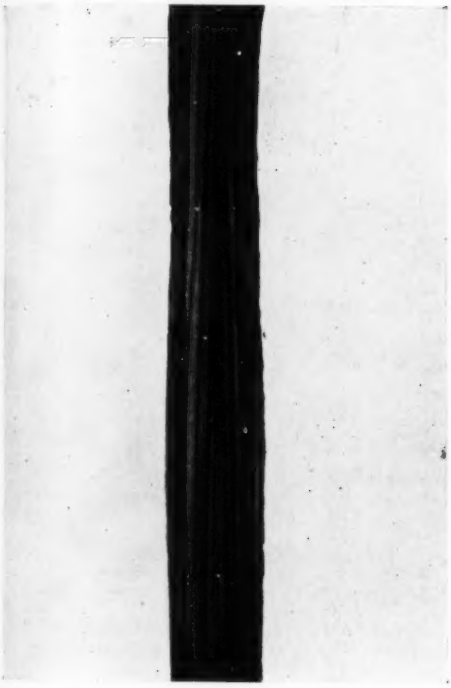

FIG. 1

FIG. 3

FIG. 2

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Explanation of Plate XIX

Fig. 1. Showing linear chlorosis in leaf. Sulphathiazol— 3 gm.

Fig. 2. Showing influence of drugs on root growth: (1) Control. (2) Sulphadiazine 1·5 gm. (3) Sulphadiazine 3 gm. (4) Sulphathiazol 1·5 gm. (5) Sulphathiazol 3 gm. (6) Sulphanilamide. 1·5 gm. (7) Sulphanilamide 3 gm. (8) Sulphaguanidine 1·5 gm. (9) Sulphaguanidine 3 gm. (10) Sulphamerazine 1·5 gm. (11) Sulphamerazine 3 gm.

Fig. 3. Showing development of multiple buds on the lower nodes. Sulphadiazine—3 gm.

"THE ATEMOYA", A PROMISING NEW ANNONA HYBRID

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The genus Annona contains several species of which about six produce edible fruits of some importance. The most commonly grown annonas in India are *A. squamosa* (sugar apple or *sitaphal*) and *A. reticulata* (bullock's heart or *ramphal*). The other annonas which have been introduced into India in recent times and grown on a very small scale are *A. cherimolia* (cherimoya) and *A. muricata* (sour sop).

A. cherimolia which is considered to be the best among all the edible annonas, is known to have originated in the highlands of tropical America and therefore does not do well when grown at sea-level in most parts of the peninsular India but it is reported to flourish to a small extent at higher elevations in the hills of South India and Ceylon. Much less commonly grown in India is *A. muricata*, which is comparatively sour in taste and is grown more in the rainy climates of Assam, lower Burma and Ceylon. Another edible species is *A. diversifolia* of Central America which has not yet been introduced into India. There is also a less known species of annona, called *A. glabra*, found growing in the eastern as well as western tropics.

Besides the above six natural species of edible annonas, altogether new hybrid forms of annonas have been evolved by crossing *A. squamosa* with *A. cherimolia*.

Hybrids between *A. cherimolia* and *A. squamosa* were first produced in Florida by P. J. Wester as far back as the years 1908 and 1910 at the Sub-tropical Garden, Miami, Florida, U.S.A., and later carried by him to the Philippines in 1911 and planted there at the Lamao Experiment Station. The hybrid plant was named by Wester as 'Atemoya', the first part of the name being derived from 'Ata' which is Brazilian name for *A. squamosa* and the second part from the second syllable of the name cherimoya. The aim of crossing these two species of annona was to develop a fruit having

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the delicious flavour of the cherimoya and yet adaptable to strictly tropical conditions.

A detailed account of the hybrid plants planted in the Philippines was published by Wester in an illustrated article " Hybridisation of annonas " in the *Philippine Agricultural Review*, Vol. VIII, Manila, 1915. In this experiment 24 hybrids were grown from seed collected from a single fruit which was the result of a cross between *A. squamosa* (pistillate parent) and *A. cherimolia* (staminate parent). Nineteen of these hybrid seedlings bore fruits in 1914. During the flowering of these hybrids Wester observed that their flowers being protogynous and entomophilous were not setting fruits satisfactorily when let alone and therefore he hand-pollinated them by obtaining pollen from other fertile species such as *A. squamosa*, *A. reticulata*, *A. cherimolia* and *A. glabra*. Very few fruits could be made to set on each hybrid plant and only 2 trees gave 47 and 26 fruits respectively, whereas on the rest of the trees the number of fruits ranged from 1–14.

The hybrid plants under study, by Wester, were found to be very similar in habit, stems, leaves and flowers to *A. cherimolia*. The leaves of hybrids were frequently twice as large as in those of the parents and besides, they had inherited the elongated shape of the leaf of *A. squamosa*. In the fruits of the hybrid seedlings, the cherimoya flavour was more or less apparent in six hybrids, the rest not having any distinctive cherimoya flavour; in quality no one was inferior to, and a number were distinct improvements upon *A. squamosa*. The carpels of fruits of three hybrids showed smoother skin with areoles slightly depressed while in the rest of the hybrids the fruits had a markedly tuberculate skin with more or less distinct protuberances on the areoles.

Referring to Wester's another hybridsing experiment at Miami, Florida, in which unlike the experiment described above, *A. cherimolia* was used as pistillate parent and *A. squamosa* as staminate parent, W. E. Safford, in the *Standard Cyclopedia of Horticulture* by L. H. Bailey observes " Very recently there has been received from Florida an interesting fruit borne by a hybrid, the result of pollinating the stigmas of a cherimoya with the pollen of *A. squamosa*. The leaves of this plant are very broad, resembling those of *A. cherimolia* in shape but glabrous like those of *A. squamosa*. The fruit resembles that of *A. cherimolia* in form but the protuberances are very distinct and covered with a glaucous bloom like that of *A. squamosa*. The seeds are distinct from both species, larger than those of *A. squamosa* and much darker coloured than those of *A. cherimolia* and the pulp is very juicy with the fine, slightly acidulous flavour of the cherimoya ".

In the accounts of the above-mentioned Wester's experiments at Miami in Florida, as well as at Lamao in the Philippines no reference is to be found about the degree of seediness or seedlessness of the fruit in different hybrids.

Concluding the results of his observations on the performance of the hybrid seedlings during 1914 at Lamao, Wester further says that the work has been disappointing so far in several respects and commercially speaking, no hybrid has yet been produced that is a success from the view-point of productivity.

Wester continued the hybridising work at Lamao in the Philippines during 1915 and also obtained successful crosses between the various atemoya mother plants and other species of annona such as *A. cherimolia*, *A. muricata* and *A. reticulata*. No further account of these hybrids seems to have been published later by Wester, but in the *Philippine Agricultural Review*, Vol. VIII of 1915, he gives a photograph of a flowering branch of a hybrid between Atemoya and *A. reticulata*.

In India, hybridisation experiments on annonas were carried out by G. B. Deshmukh at the Ganeshkhind Fruit Experiment Station, Poona, in the year 1927. In his experiment, Deshmukh used *A. cherimolia* as female and *A. squamosa* as male parent (Plates 1–4) and crossed their flowers in July 1927 and obtained only one mature hybrid fruit in February 1928. Ten seeds collected from this fruit were sown in earthen pots on 22nd February and they all germinated about 19th March 1928. The ten hybrid plants were nursed in a plant nursery for two years and were subsequently transplanted in their permanent quarters in January 1930. The records maintained at the Experiment Station show that only six out of the original ten plants survived and all the six flowered for the first time in 1933. Between the years 1933 and 1949, no individual tree performance record of these hybrid trees was maintained in the garden register, probably due to Deshmukh's retirement from his post at the Experiment Station in 1930 though casual notes and descriptions of fruits of hybrids Nos. 1 and 3 were made by the then officers in-charge of the Experiment Station, in later years. During this long period of 15 years the trees seem to have been allowed to grow and fruit indiscriminately. For a number of years they remained crowded around and partially shaded by other botanical trees. In 1948, the hybrid trees were, however, reclaimed from the unfavourable surroundings and given good cultural treatment. Since 1949 regular observations on their growth and yield were recorded by the present writers of this article. Also a close study of the morphological characters of each hybrid was made with

a view to finding out its variation from or similarity to the characters of either parent. The study has resulted in the evolution of a promising hybrid No. 2, which is a distinct improvement upon both parent annonas and is also markedly superior to other sister hybrids. It yields a fair crop of about 80 large-sized fruits without any hand pollination. The fruit of this hybrid has abundant seedless pulp and excellent cherimoya taste and flavour.

An account of only five hybrids out of the six is given in this article as the hybrid No. 4 has remained stunted and has never set any fruit since it was planted, though it has been regularly flowering.

The following are the morphological descriptions of the parents *A. cherimolia* (female) and *A. squamosa* (male) and the five hybrid plants under study.

Annona cherimolia (CHERIMOYA)

The cherimoya used as female parent in this experiment is a small, semi-deciduous, spreading tree growing to a height of about 28′.

The leaves are elliptical, 7″ long, 2·5″ broad, shortly acuminate at the apex and acute at the base. The upper surface is sparsely hairy and the lower velvety-tomentose.

The shape of fruit is sub-globose. The stalk is thick, stout and squarely set in a round disc-like depression. The skin which is pale green, thin and pliable has a rough surface covered with deep, U-shaped areoles resembling finger-prints in wax. The areoles near the base, are longer, more closely spaced and have tiny circular wart-like tubercles within their rounded apices. The areoles in the middle of the fruit are deeper. Towards the apex, the U's of the areoles are much shallower, devoid of warts and are rather irregular in outline. The flesh carpels within the fruit are not connected with the external areolar divisions of the skin which presents a whole contiguous unbroken surface unlike the tuberculate and distinctly divided skin of *A. squamosa*. Between the skin and the pulp there is a layer of a sandy, gritty, pink-coloured, tasteless substance to a thickness of 5–10 mm. The layer of this substance is not uniformly thick and sometimes it is found to penetrate deep into the regular pulp thus affecting its taste adversely.

The pulp is more or less a homogeneous mass of closely cohering carpels, which cannot be easily separated. Over half the number of carpels are seedless. The ratio of seeded to seedless carpels is 1 : 1·5. The pulp in well-ripened fruits is extremely delicate, melting, sweet, juicy and has an appealing acidulous flavour. The flesh around the seeds in the case of seeded carpels is as tender or melting as that of the seedless carpels. Seeds are 15 mm. long, 9 mm. broad, dark-brown, and flat.

Usually, an average cherimoya fruit of good size, about 4¼″ long and 3½″ in transverse diameter, weighs 10–12 oz. and contains about 45 seeds. The seedlessness of over half the number of carpels is, however, the most redeeming feature of the pulp of cherimoya.

Although cherimoya grows fairly well at Poona, the most disappointing feature about its cropping habit is that the fruits do not ripen uniformly, the pulp often tending to become hard and lumpy.

The cherimoya at Poona, flowers from June to September and yields mature fruits from November to February.

A 35-year old cherimoya tree produces on an average 180 fruits annually.

Annona squamosa (SUGAR APPLE OR SITAPHAL)

Annona squamosa used as the male parent in this experiment, is a small semi-deciduous, spreading shrub, about 15–20′ high.

The leaves are elliptic, shortly acuminate at the apex and acute at the base, about 4–5″ long, 2″ broad, green and glabrate.

A *sithaphal* fruit of good size, about 3·5″ long and 4″ in transverse diameter weighs about 10 oz. The shape of the fruit is cordiform (heart-shaped). The skin is tuberculate, having distinct polygonal, carpellary protuberances. The protuberances at the base of the fruit, around the stalk are comparatively more elongated than polygonal. The surface of the skin is yellowish green and covered with whitish bloom.

When the fruit ripens, the skin cracks at several places and breaks into individual tubercles, each external tubercle representing a corresponding fleshy carpel within the fruit. The tubercles forming the skin of the fruit are boat-shaped and hollow internally and contain a sort of pinkish gritty matter. This gritty matter is restricted to the tubercle only and does not penetrate into the pulp.

The pulp is thin, off-white in colour, custard-like, of delicate texture and sweet. It is composed of loosely cohering, short, broad, conical fleshy carpels, each of which normally contains a darkish bronze-coloured, bean-like seed which has a halo-like streak on the testa. A few carpels may be seedless. The ratio of seeded to seedless carpels is 3: 1. Seeds are 16 mm. long and 7 mm. broad. Normally an average sized fruit contains about 50 seeds.

A. squamosa flowers at Poona from March to September and ripens its fruits from August to November. The flowers produced during the hot weather, between March and April, are generally abortive, but the flowers

produced with the setting in of the south-west monsoon in June generally set into fruits.

ANNONA HYBRID NO. 1

Leaf Characters

Leaf elliptic, acute at base and apex, glabrate, 6″ long, 2·2″ broad.

Fruit Characters

Form.—Variable, ovoid to flat spheroid in shape; base and apex broadly rounded; stalk fairly thick and squarely set into a shallow depression at the base.

Size.—Small to medium, 3″ long and 3·4″ in transverse diameter; weight 8–10 oz.

Skin.—Thin, ashy green with glaucous bloom; divided into contiguous, flat tubercles of varying size and shape; tubercles near the base and on one side of the stalk very tiny and on the other side longish and flat; apex almost smooth with polygonal flat tubercles. While removing the pulp, the skin does not break into individual tubercles carrying their own flesh carpels as in *A. squamosa* but maintains its entirety as in *A. cherimolia.*

Pulp.—Creamy white; flesh carpels longish, pyramidal, delicate in texture, fairly closely cohering, not easily separable; ratio of seeded to seedless carpels 1 : 5; taste and flavour of seedless pulp pleasantly sweet, acidulous and melting and those of seeded pulp, a little coarser in texture and devoid of flavour; seeds light bronze in colour, plump 16 mm. long and 9 mm. broad, full, elongate to oval in shape, about 13 in number.

Flowering and fruiting.—The season of flowering is from April to July, but generally the flowers produced in the hot weather from April to June are abortive. The setting of fruits takes place from July to September and mature fruits are available in October and November and sometime upto January.

About 10 fruits could only be gathered annually from this tree.

Parent Characters Inherited by Hybrid No. 1

Characters showing affinity to A. cherimolia.—(1) Contiguous nature of the under-skin. (2) Seedlessness of most of the carpels. (3) Acidulous, pleasant flavour of pulp. (4) Closely cohering fleshy carpels.

Characters showing affinity to A. squamosa.—(1) The skin tends to be tubercular. (2) Pulp can be completely scooped out of the skin, leaving no gritty matter behind.

ANNONA HYBRID NO. 2

Leaf Characters

Leaf elliptic, base acute, apex shortly acuminate, glabrate, 7·5″ long, 2·7″ broad.

Fruit Characters

Form.—Commonly cordiform; base broad, expansive with broad shoulders; apex generally broadly rounded or sometime narrowly rounded; stalk very thick, stout and squarely set into a deep depression formed in the base by the bulging sides of the fruit.

Size.—Large about 3·5″ long and 4·5″ in transverse diameter, average weight 12 oz. and the maximum weight reaching upto 30 oz.

Skin.—Very thick; dark green; divided into distinctly separate, non-contiguous and highly out-projecting tubercles; tubercles near the base and around the stalk very large, closely spaced, massive, lobulate and pendulous with rounded apices; in the middle of the fruit, the tubercles are smaller and more roundish and towards the apex, they again become heavy, massive and pendulous; areoles disjoining the tubercles remain imperceptible in an immature fruit but they become widened and develop a pinkish bloom when the fruit ripens; though tubercles over the surface of the fruit are non-contiguous and are distinctly separate individuals, they remain attached to a contiguous under-skin which holds them together; when the fruit ripens, the skin does not crack and break into individual tubercles carrying their own fleshy carpels as in *A. squamosa*, but maintains its entirety as in *A. cherimolia*. The tubercles forming the exterior skin of the fruit are hollow internally and contain a sort of pinkish gritty matter.

Pulp.—Creamy white; fleshy carpels long, flat, large, very delicate in texture, very closely cohering, not easily separable; ratio of seeded to seedless carpels on an average is 1 : 6 to 1 : 23. Between the skin and pulp, there is a layer of gritty, sandy, pinkish, tasteless substance upto a thickness of about 2 millimeters. The pulp which is composed of fleshy carpels is creamy white in colour, pleasantly sweet, acidulous, aromatic, extremely delicate, mellow and melting; seeds light bronze in colour with distinct light-coloured longish streaks on the testa, 15 mm. long and 10 mm. broad, flat, about 3–28 in number, the average number being about 10.

Flowering and fruiting.—The season of flowering is from April to June or July; but generally the flowers produced in the hot weather from April to early June are abortive. The setting of fruits takes place from July to

September and mature fruits are available from October to January. On an average, the tree yields about 50–80 fruits annually.

Parent Characters Inherited by Hybrid No. 2

Characters showing affinity to A. cherimolia.—(1) Contiguous nature of under-skin. (2) Seedlessness of most of the carpels. (3) Presence of gritty, pinkish matter between skin and pulp. (4) Inseparable cohering fleshy carpels presenting almost a homogeneous mass of pulp matter. (5) Highly acidulous and aromatic flavour of the pulp.

Characters showing affinity to A. squamosa.—(1) Distinctly tuberculate skin with raised and projecting tubercles. The tubercles are not however polygonal as those of *A. squamosa*. (2) Seeds have haloed streaks.

Annona Hybrid No. 3

Leaf Characters

Leaf elliptic, acute at base and apex, glabrate, 6·5″ long, 2·5″ broad.

Fruit Characters

Form.—Ovoid to spheroid; base broad with expansive bulging sides which gradually taper into a broadly rounded apex; stalk very stout and thick and set squarely into a deep depression in the base.

Size.—3½″ long, 3¼″ in transverse diameter and about 10 oz. in weight. Large fruits may weigh upto 15 oz.

Skin.—Thick, light green in colour, warty; divided into fused, contiguous tubercles and areoles; around the stalk and reaching the middle of the fruit are numerous, closely spaced, longish, protruding and pendulous tubercles, the apices of which are slightly pulled upwards from the general surface of the skin; the lower half of the skin is covered by broad and deep crescent-shaped areoles, the apices of which bear wart-like tubercles; on removing the pulp, the skin does not break into individual tubercles carrying their own flesh carpels as in *A. squamosa* but maintains its entirety as in *A. cherimolia.*

Pulp.—Creamy white; flesh carpels so closely cohere that the pulp presents one homogeneous mass; between the skin and pulp there is a layer of pinkish gritty matter upto a thickness of 3–4 mm. The pulp which is composed of fleshy carpels is fairly pleasant, sweet, acidulous and has good cherimoya flavour. The pulp of seeded carpels is comparatively more fibrous than the pulp of seedless carpels; ratio of seeded to seedless carpels varies from 1:3 to 1:5; seeds are shining dark bronze in colour, 16 mm. long, 7 mm. broad, slightly depressed sides with thin testa, about 25 in number.

Flowering and fruiting.—The season of flowering is from April to September. Flowers produced in the hot weather between April and June are generally abortive. The setting of fruits takes place by July to September and mature fruits are available from October to January.

On an average the tree yields 10–30 fruits annually.

Parent Characters Inherited by Hybrid No. 3

Characters showing affinity to A. cherimolia.—(1) Contiguous nature of skin with U-shaped areoles on the lowerhalf. (2) Seedlessness of a good number of carpels. (3) Presence of a gritty pinkish matter underneath the skin. (4) Cohering carpels cannot be easily separated and they present almost a homogeneous mass of pulpy matter. (5) Highly acidulous flavour of the pulp.

Characters showing affinity to A. squamosa.—The skin is partially tuberculate at the base of the fruit around the stalk.

ANNONA HYBRID NO. 5

Leaf Characters

Leaf narrowly elliptic, base acute, apex shortly acuminate, glabrate 5·8″ long, 2·1″ broad.

Fruit Characters

Form.—Uniformly cordiform, regular in outline, sides bulge equally all around and taper towards a narrowly rounded apex. Stalk thin and squarely set into a very shallow depression in the base.

Size.—Small 2·5″ long and 2·5″ in transverse diameter, about 6–10 oz. in weight.

Skin.—Papery thin, velvety smooth, ashy green, covered by a uniformly designed network of contiguous, tiny, flat, polygonal, overlapping tubercles which are smooth and soft to the touch. The tubercles near the base and around the stalk are longish, crowded and slightly raised but they get more polygonal and flatter towards the middle and apex, giving the fruit a very smooth appearance. The skin being extremely thin gets easily ruptured irregularly when the fruit ripens; on removing the pulp, the skin does not break into individual carpels but maintains its entirety as in *A. cherimolia.*

Pulp.—White, delicate, aromatic, translucent, thin, slightly acidulous, melting, and pleasantly sweet; fleshy carpels flat, big and juicy. Seed is seen through the white translucent pulp. Flavour, taste and delicacy of pulp resemble those of *A. squamosa.* Fleshy carpels despite their delicate texture can be easily separated from one another; ratio of seeded to seedless

carpels is 1 : 2; seedless carpels, though greater in number being extremely thin, do not contribute appreciably to the bulk of the pulp which apparently presents a seedy appearance; seeds dark bronze in colour, 15 mm. long and 7 mm. broad, angular, thin, longish, with delicate, soft testa; about 60 in number.

Flowering and fruiting.—The season of flowering is from April to September. Flowers produced in the hot weather between April and June are generally abortive. The setting of fruits takes place by about the middle of July and upto September and mature fruits are available from November to January.

On an average the tree yields 7–30 fruits annually.

Parent Characters Inherited by Hybrid No. 5

Characters showing affinity to A. cherimolia.—Contiguous nature of under-skin.

Characters showing affinity to A. squamosa.—(1) Tuberculate skin. (2) Shape of the tubercles uniformly polygonal. (3) Translucent nature of the pulp of the carpel showing the seed within it. (4) Flavour and texture of the flesh are those of *A. squamosa*. (5) Fleshy carpels are easily separable. (6) Uniformly cordiform shape of the fruit.

ANNONA HYBRID NO. 6

Leaf Characters

Leaf elliptic, lanceolate, base and apex acute, glabrate, 6·5″ long and 1·9″ broad.

Fruit Characters

Form.—Irregularly spheroid due to sporadic swellings of tubercles around the base of the fruit. Stalk squarely set in a shallow depression; apex broadly rounded.

Size.—3·2″ long, 3″ broad. Small to medium, weight 6–8 oz.

Skin.—Very thin, ashy green, slightly rough with fused flat tubercles which are longish and narrow at the base, tiny and roundish in the middle and much larger, flatter and more polygonal towards the apex. The skin is brittle and cracks irregularly on ripening. The skin does not break into individual tubercles carrying their own fleshy carpels as in *A. squamosa*, but maintains its entirety as in *A. cherimolia*.

Pulp.—White, coarse, seedy, with tightly cohering fibrous flesh carpels which cannot be separated easily; Pulp around the seeded carpels is very

tough and fibrous; on the whole the consistency of the pulp is curdy, mushy, and mixed up; taste is sweet, and flavour acidulous. The ratio of seeded to seedless carpels is 1 : 1; seeds light bronze-coloured; 16 mm. long, 7 mm. broad, full, sometimes flat with a fairly hard testa; 20–35 in number.

Flowering and fruiting.—The season of flowering is from April to end of June. Flowers produced in the hot weather between April and May are generally abortive. The setting of fruits takes place by about the end of June, *i.e.*, after the setting in of the monsoon, continues upto September and mature fruits are available from November to January.

On an average the tree yields 50–150 fruits annually.

Parent Characters Inherited by Hybrid No. 6

Characters showing affinity to A. cherimoya.—(1) Contiguous nature of the under-skin. (2) Seedlessness of nearly half the number of fleshy carpels. (3) Tightly cohering fleshy carpels presenting a homogeneous mass of pulp. (4) Acidulous flavour of pulp.

Characters showing affinity to A. squamosa.—(1) Tuberculate skin. (2) Skin cracks on ripening.

SUMMARY

1. All hybrids have inherited the glabrate leaf character of *A. squamosa* as opposed to the tomentose character of the leaf of *A. cherimolia.*

In size the leaves of hybrids are comparatively bigger than those of *A. squamosa* but are almost as large as those of *A. cherimolia.*

The leaves of three hybrids (Nos. 1, 2 and 3) are almost similar in shape and size to those of *A. cherimolia.* The leaves of No. 5 are a little shorter while those of No. 6 have narrower lamina.

2. The flowering and fruiting seasons of hybrids coincide with those of *A. squamosa.*

3. All hybrids differ from one another in the external and internal structure of their fruits. Skin, pulp and seed characters of both parents seem to have been inherited in varying degrees by each hybrid.

The shape of fruit of Nos. 2 and 5 hybrids is regularly cordiform like that of *A. squamosa.* In No. 5, the carpels are flat but uniformly polygonal as those in A. *squamosa,* while in No. 2 the carpels are tuberculous, *i.e.*, very prominently projecting outwards and drawn out. In No. 3, the surface of the skin is areolar in the middle and towards the apex like that in *A. cherimolia* and

in Nos. 1 and 6 the exterior shape of carpels is rather undefined but the surface of the skin, generally tends to be tuberculous like that in *A. squamosa.*

The skin in all hybrids, irrespective of its tuberculous or areolar character, is contiguous like that in *A. cherimolia.*

The structure of pulp, taste and flavour of fruits of hybrids Nos. 1, 2, 3 and 6 are to a considerable extent like those of *A. cherimolia* while in these respects No. 5 is more akin to *A. squamosa.*

The streaked character of the seed of *A. squamosa* is distinctly noticeable in the seed of No. 2 hybrid, but not in any other hybrid.

Seedlessness is induced in varying degrees in the fruits of different hybrids. Hybrids Nos. 1, 2 and 3 have much greater number of seedless carpels than *A. cherimolia.* No. 6 is as good in seedlessness as *A. cherimolia.* No. 2 has the fewest seeds and No. 5 is most seedy among the hybrids under study.

The texture of flesh of seeded carpels is coarser and more fibrous than that of seedless carpels in hybrids Nos. 1, 3 and 6 in contrast to delicate and melting flesh of seeded carpels in either parent.

Seeds of different hybrids slightly differ in shape, size and shade of bronze.

In eating quality No. 2 is excellent. It has sweet, delicate, melting, acidulous and aromatic pulp which is almost seedless. Nos. 1 and 3 are only second to No. 2 in taste, flavour and texture of pulp. No. 5 is a very seedy fruit, having squamosa taste and flavour and No. 6 has a mixed curdy taste and flavour which are inferior to those in *A. cherimolia.*

(4) In cropping ability hybrids Nos. 1, 3 and 5 are extremely poor, bearing 10, 30, 30 fruits respectively. No. 2 bears a maximum crop of about 80 fruits and No. 6 bears as many as 150 fruits in the most favourable season. Some of the fruits of the hybrid No. 2 were twice as large as either parent.

Conclusion

The hybridisation has resulted in the development of hybrid No. 2 which is a distinct improvement on either parent from the point of seedlessness and eating quality. It is also capable of yielding a fair crop of about 80 fruits annually (unaided by hand-pollination) which can considerably be raised by making cross-pollination possible between it and *A. squamosa.* This can be arranged by growing a few plants of *A. squamosa* along with the plants of hybrid No. 2 in the same plantation.

The poor setting of fruits generally observed in all hybrids confirm the observations of P. J. Wester that the flowers of atemoyas require to be cross-pollinated for setting a successful crop. According to Wester's investigations carried out in Florida, U.S.A., the flowers of annonas are protogynous and entomophilous, *i.e.*, the pistils of the flowers are receptive before the anthers have developed ripe pollen, cross-pollination being therefore necessary and some outside agency like insects being required to effect it.

To overcome this problem of cross-pollination, adequate provision for keeping a sufficient number of bee-hives should be made for the mixed plantations of Atemoyas and *A. squamosa*, that may be planted in future.

As the progeny raised from seed would not breed true to type, No. 2 hybrid should be further propagated either by layering or by inarch-grafting on the root-stocks of *A. squamosa* or *A. cherimolia*.

ACKNOWLEDGEMENTS

The authors are highly indebted to Professor L. S. S. Kumar, Principal, College of Agriculture, Poona, for going through the manuscript and for offering valuable suggestions and criticism. Grateful thanks are also due to Shri P. S. Haldankar, Artist to the Department of Agriculture, Bombay, and Shri V. V. Rao, Lecturer in Botany, College of Agriculture, Poona, for preparing the photographs appearing in this article.

EXPLANATION OF PLATES

PLATE XX

FIG. 1. *Annona squamosa* (*sitaphal*), staminate parent.
FIG. 2. *Annona squamosa* (*sitaphal*) cut open to show internal structure.

PLATE XXI

FIG. 3. *Annona cherimolia* (*cherimoya*), pistillate parent.
FIG. 4. *Annona cherimolia* (*cherimoya*) cut open to show internal structure.

PLATE XXII

FIG. 5. The five atemoyas (annona hybrids) under study. Upper row, from left: Nos. 1, 2 and 3 hybrids. Lower row, from left: Nos. 5 and 6 hybrids.

PLATE XXIII

FIG. 6. Hybrid No. 2.
FIG. 7. Hybrid No. 2 cut open to show internal structure.

PLATE XXIV

FIG. 8. Leaves of *Annona cherimolia* and *Annona squamosa*.

PLATE XXV

FIG. 9. Leaves of five hybrids under study. Upper row, from left: Leaves of hybrids 1, 2 and 3. Lower row, from left: Leaves of hybrids 5 and 6.

PLATE XXVI

FIG. 10. Twigs of *A. cherimolia* and *A. squamosa*.
FIG. 11. Twigs of five hybrids under study.

FIG. 1

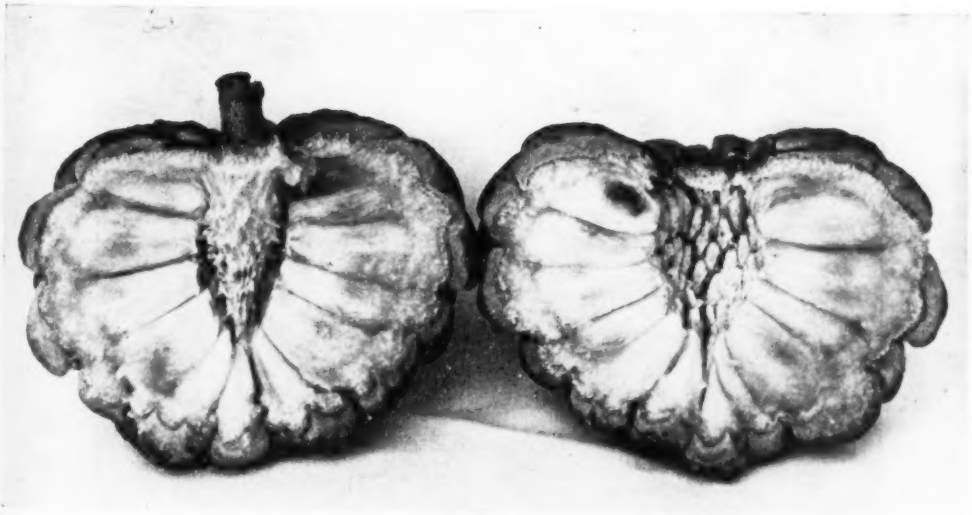


FIG. 2

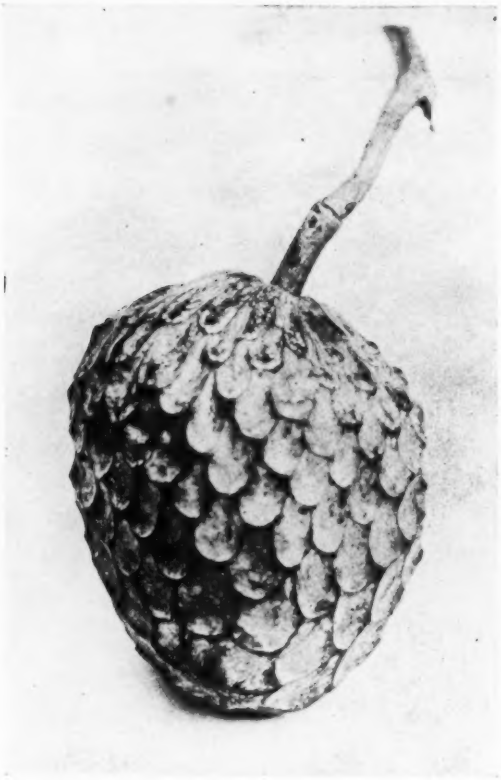


FIG. 3

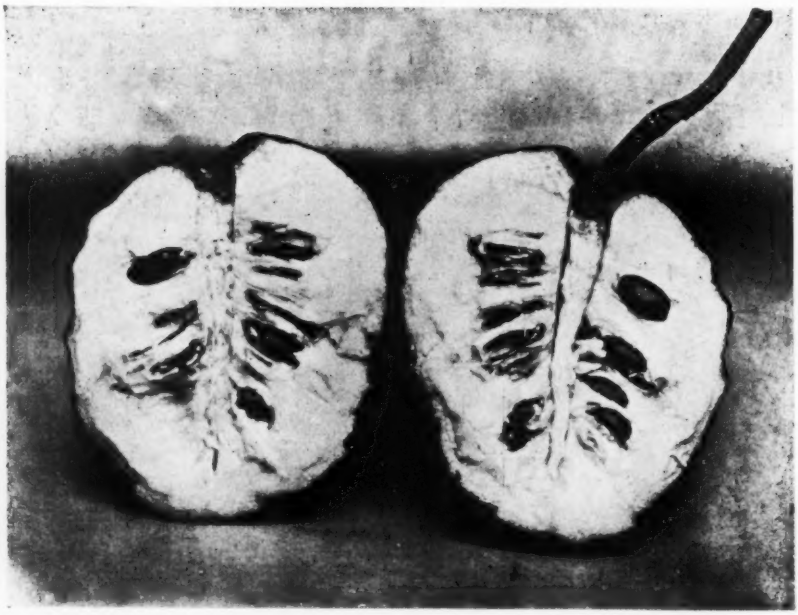


FIG. 4

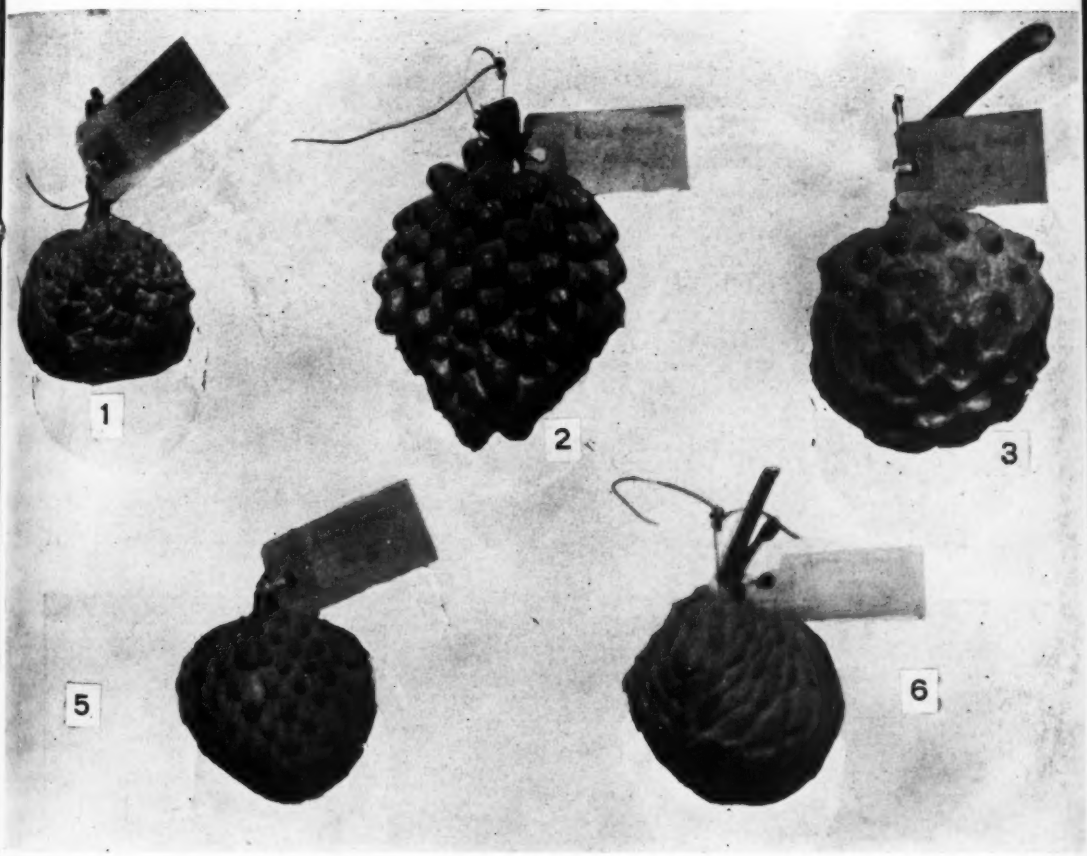


FIG. 5

FIG. 6

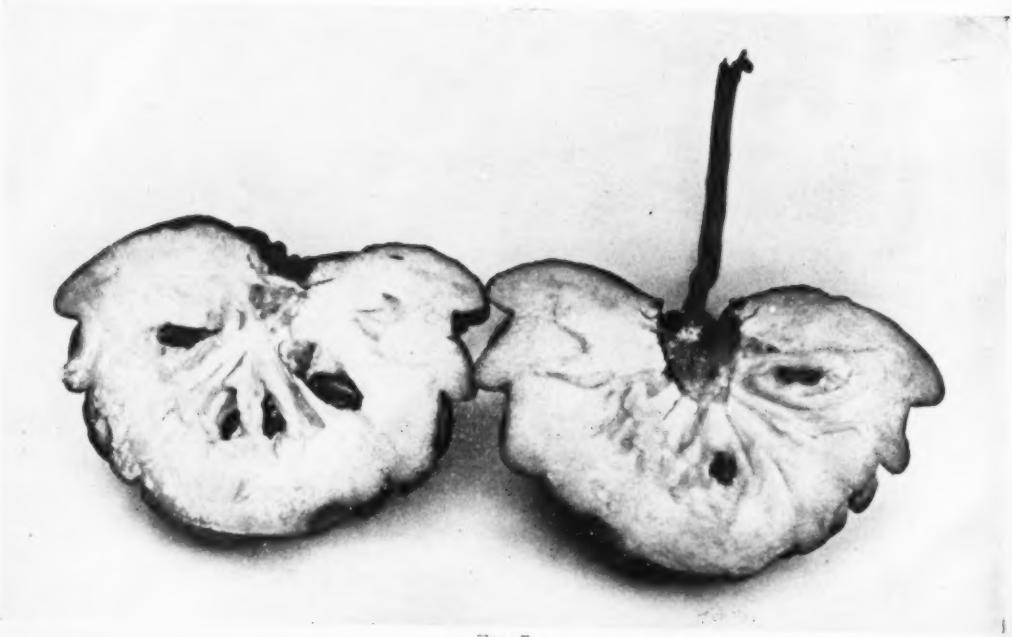
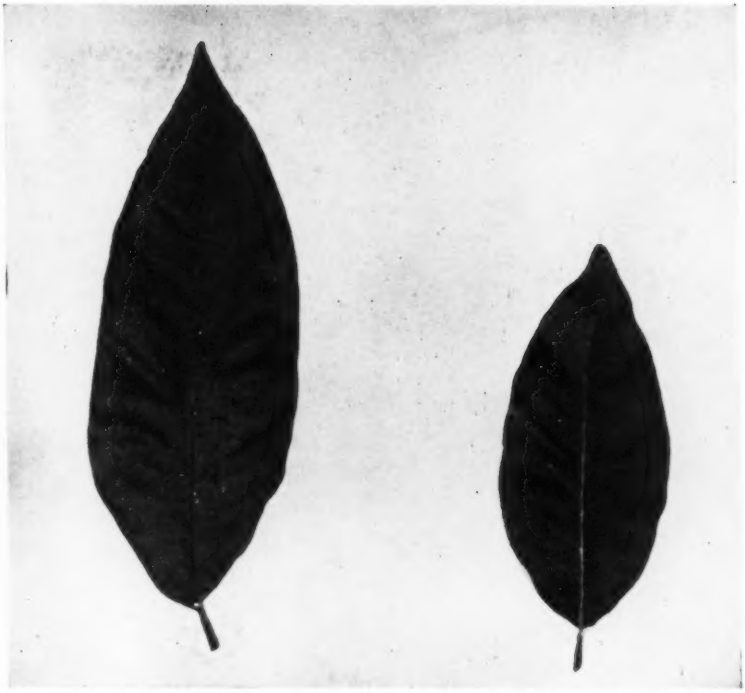


FIG. 7



A. CHERIMOLIA A. SQUAMOSA

FIG. 8

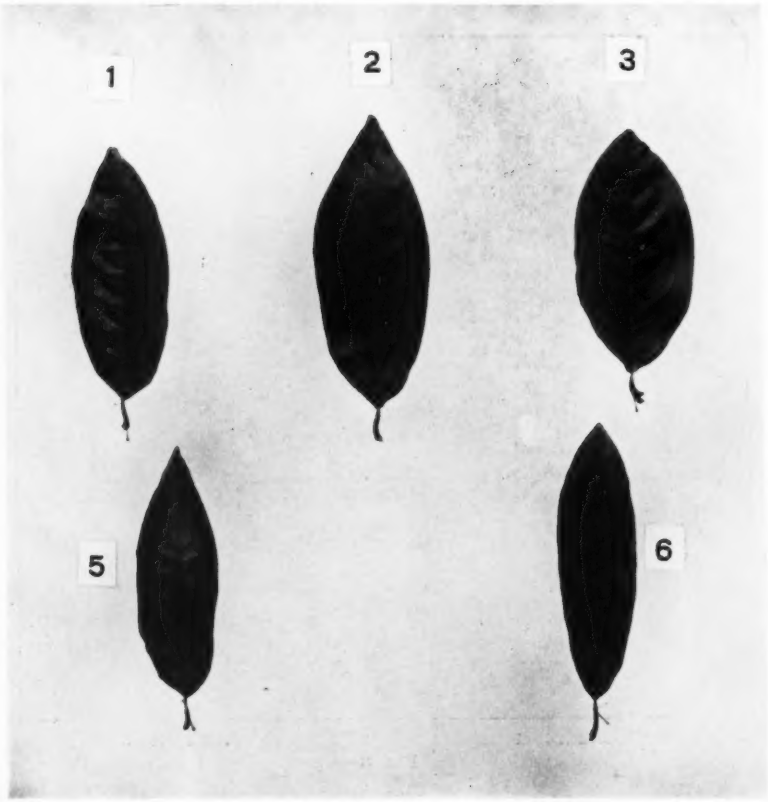


FIG. 9

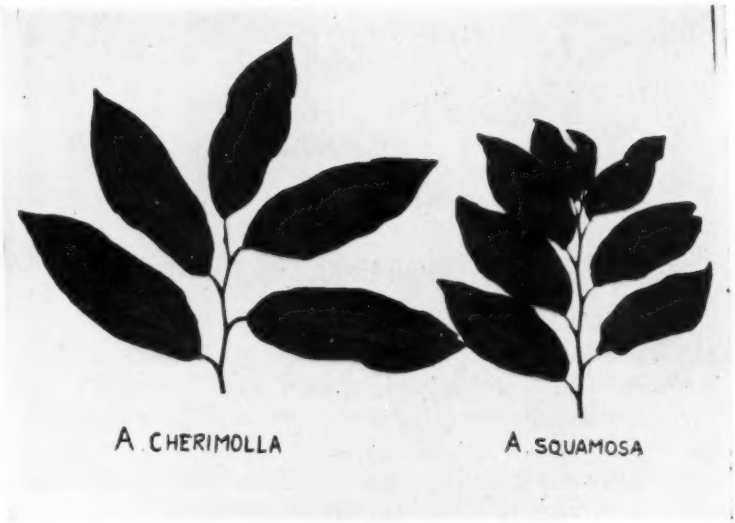


FIG. 10

FIG. 11

SEED ABORTION IN *ELYMUS-SITANION* ALLOPOLYPLOIDS

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Introduction

Elymus-Sitanion hybrids.—The chromosome numbers of the three parental species *Elymus glaucus*, *Sitanion hystrix* and *Sitanion jubatum* was determined as $2n = 28$ by Stebbins and Love (1941). Cytogenetic investigations in the F_1 hybrids from the two crosses *Elymus glaucus* × *Sitanion jubatum* and *Elymus glaucus* × *Sitanion hystrix* were carried out by Stebbins, Valencia and Valencia (1946), who observed regular meiotic behaviour in the microsporocytes of the F_1 hybrids of the two crosses. Nevertheless, the hybrids were found to be completely sterile. On the other hand, Stebbins and Vaarama (1952, 1954) obtained highly fertile allopolyploids by doubling the chromosome number of the sterile hybrids. In the C_2 generation (second generation of the colchicine doubled F_1 diploid hybrid) there was a considerable range of seed setting and pollen fertility. Furthermore, there seemed to be no good correlation between seed setting and stainable pollen. Therefore, the studies presented in this paper, which consist of cytological examination of the development of the embryo-sac, embryo and endosperm, were begun with the idea of determining the causes of seed collapse in the allopolyploids from the two crosses *Elymus glaucus* × *Sitanion jubatum* and *Elymus glaucus* × *Sitanion hystrix*.

Material and Methods

The material used in this study was derived from that investigated by Stebbins and Vaarama (1954). It consisted of two intergeneric crosses *Elymus glaucus*, Buckl. × *Sitanion jubatum*, J. G. Sm. and *Elymus glaucus*, Buckl. × *Sitanion hystrix* (nutt) J. G. Sm. The F_1 hybrids were completely sterile but a number of cuttings treated with colchicine gave a series of fertile amphidiploid sectors (C_1 generation). The present material of these hybrids was limited to seven C_2 plants produced by selfing different C_1 plants. Table I gives the strains used in each cross. The names of the strains represent

* This paper forms a part of the thesis submitted in partial fulfilment of the requirement for the Degree of Doctor of Philosophy of the University of California.

the places of their collection. To avoid confusion new numbers are given to the C_2 clones. Finally the allopolyploids derived from the cross *E. glaucus* × *S. jubatum* will be referred to as *glaucus-jubatum* and those from the cross *E. glaucus* × *S. hystrix* as *glaucus-hystrix*.

The hybridization techniques used were the same as those described by Stebbins and Tobgy (1944) and the methods of colchicine treatment were those given by Stebbins and Vaarama (1954). Ovaries of the female parent, F_1 hybrids and allopolyploid plants were collected several times during the periods from pollen tetrads up to anthesis and at definite stages after pollination. Most of the material was collected during the summer months of 1952 and 1953. The ovaries for post-fertilization studies were collected at intervals of one day each, beginning with the day of opening of the florets to the 15th day after pollination. They were prefixed in Carnoy's fluid for 10–15 minutes and then fixed in Randolph's modification of Navashin's fluid for 24 hours as given by Johansen (1940). The material was washed three or four times in 70% ethyl alcohol, passed through the dehydration series of butyl alcohol, and embedded in paraffin. The sections of 12–22 microns in thickness were stained with Stockwell's variation of Flemming's triple strain.

TABLE I

The material under investigation

Parental strains used in the cross	F_1 diploid hybrid	Numbers of C_2 clones Old	New
(1) *E. glaucus* × *S. jubatum*			
Santa Paula × Caliente		1104–5	1
		1104–32	2
		1104–33	3
		1104–37	4
(2) *E. glaucus* × *S. hystrix*			
(*a*) Sebastopol × Fort Churchill ..	107	241–3	5
		241–5	6
(*b*) Santa Paula × Fort Churchill ..	111	243–4	7
(*c*) Knight's Ferry × Fort Churchill ..	112	..	..

RESULTS

In the two crosses *E. glaucus* × *S. hystrix* and *E. glaucus* × *S. jubatum* reported in this paper, *E. glaucus* was used as the female parent in which the

embryo-sac formation (Fig. 1) and seed development was normal. However, the development of the seed was abnormal in the diploid F_1 hybrids 107, 111 and 112 of the cross *E. glaucus* × *S. hystrix* and in the clones 1, 2, 3, 4, 5, 6 and 7 of the two *Elymus-Sitanion* allopolyploids.

Seed development in the diploid hybrids.—Cytological examination of 70 ovules of the three F_1 diploid hybrids (107, 111 and 112) from the cross *E. glaucus* × *S. hystrix* was made at several stages of development before anthesis. Of the 70 ovules studied, 29 showed the normal formation of megaspore-mother-cell stage (Fig. 2). From the detailed information given below, it is very probable that these 29 ovules in normal megaspore-mother-cell stage would have aborted at some later stage of macrogametogenesis.

In the remaining 41 ovules observations were made regarding the abnormal conditions causing seed abortion in the diploid hybrids of the cross *E. glaucus* × *S. hystrix*. In five ovules the nucellus was partially degenerated (Fig. 3) and in such ovules parts of the structures constituting the egg-apparatus, unfused polar nuclei and the antipodals were observed. Nevertheless, these structures in the five ovules did not appear normal and showed signs of gradual deterioration. In 36 of the 41 ovules the nucellus and its contents were completely degenerated (Figs. 4 and 5). From the development of the two integuments surrounding the nucellus and from the size of 36 ovules, it is suggested that their deterioration probably occurred after completion of the third mitotic division in macrogametogenesis.

Among the 70 ovules examined, none were at the correct stage for showing the two meiotic divisions, but the appearance of the 41 ovules suggested that the two meiotic and three mitotic divisions had occurred in the ovules of the three F_1 diploid hybrids. These ovules probably aborted at stages after the completion of the mitotic divisions and before the embryo-sacs completed their development to full maturity.

Observations made on the ovules of the three diploid hybrids, from anthesis onwards, indicated that the dark staining tissue of the degenerated mass that was observed in the ovules at stages before anthesis, gradually began to decrease in volume after fifth day of the opening of the florets (anthesis). However, in the case of one hybrid (112) a peculiar proliferation of cells of the outer integument was observed. This overgrowth or hyperplasia of the external integument began at anthesis and ceased about seven days later, giving to the ovule the appearance of a false caryopsis, which, of course, was completely nonfunctional.

Seed development in Elymus-Sitanion allopolyploids.—In Tables II and III the ovules of the two allopolyploid hybrids have been classified into

normal and abnormal, although, as will be mentioned below some of the ovules classified as normal did have some irregularities. The development of the normal embryo-sacs of the two allopolyploids is similar to that in the diploid parent, although in the allopolyploids, not all of them were fertilized. The number of fertilized ovules in each of the clones is given in the first row of Tables IV and V and that of the unfertilized ovules in the second row. The percentages of unfertilized ovules in the two allopolyploids is quite different, 40 per cent. in *glaucus-jubatum* and 28 per cent. in *glaucus-hystrix*. In most of the unfertilized ovules, there was no evidence of pollen grains adhering to the stigmatic surface in spite of the fact that an abundance of pollen was available.

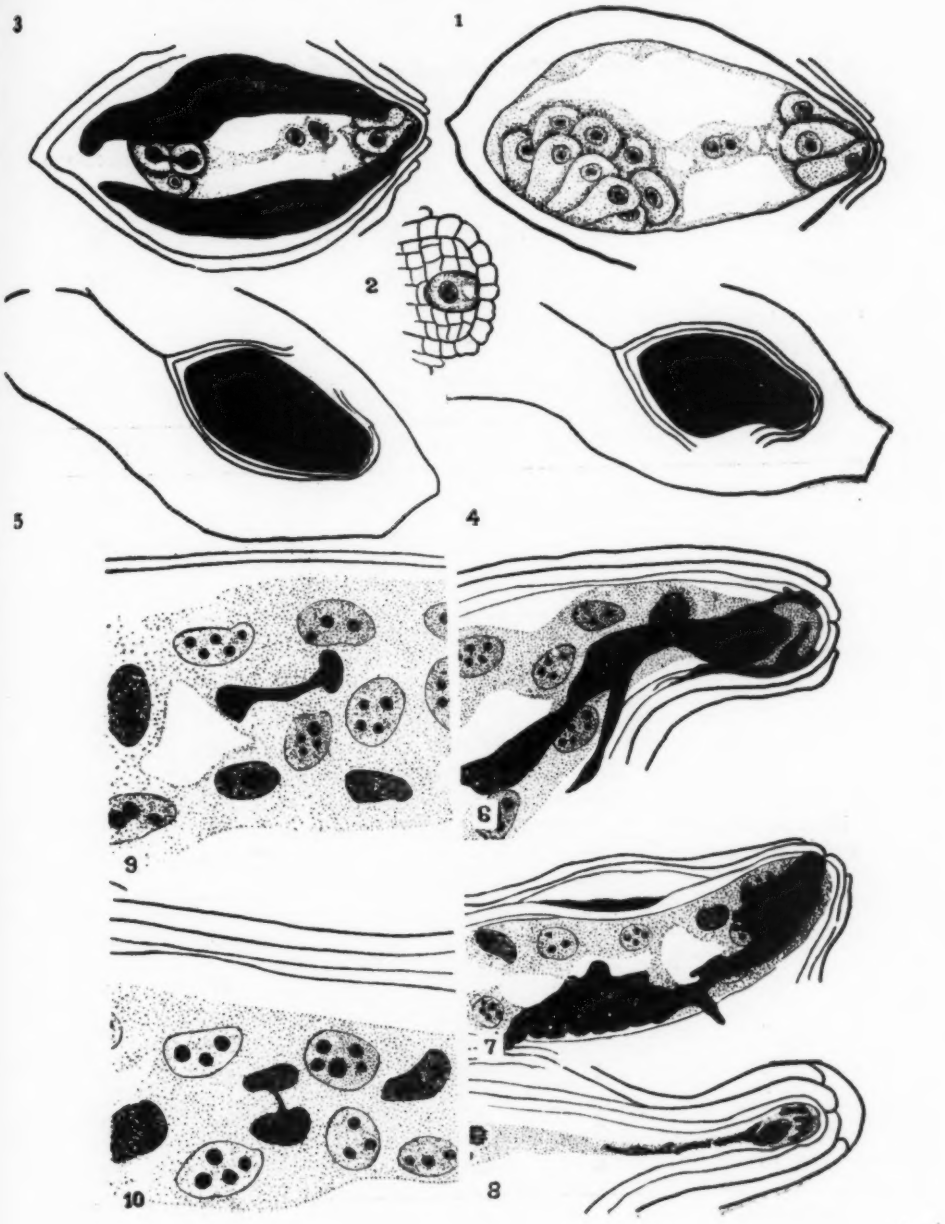
TABLE II

Fertility data of four allopolyploid derivatives of the cross E. glaucus × S. jubatum

Clone Number	1	2	3	4	Frequency	Percentage
Normal Ovules Fertilized	53*	17	7	18	95	34
Unfertilized	81	11	17	9	118	40
Abnormal Ovules Fertilized	36	19	14	1	70	26
Unfertilized	..	..	..	..	..	
Total ..	170	47	38	28	283	(100)
Normal Fertilized Ovules ..	31	36	18	64		34
Seed Set	20	31	24	48		22
Normal Pollen	51	40	5	12		39

* Except for total in the last column, figures above the mid-line are frequencies and below the mid-line are percentages.

It appears, therefore, that the stigmas of these unfertilized ovules were either not receptive or immature at anthesis. Furthermore, in the unfertilized ovules first there was a delay in the disappearance of the nucellar tissue and second the antipodals seemed to be smaller and fewer in number. These unfertilized ovules aborted 10–12 days after anthesis and they accounted for a part of the seed abortion in the two allopolyploids.



FIGS. 1–10

TABLE III

Fertility data of three allopolyploid derivatives of the cross E. glaucus × S. hystrix

Clone Number	5	6	7	Frequency	Percentage
Normal Ovules					
Fertilized	10*	42	41	93	36
Unfertilized	1	65	6	72	28
Abnormal Ovules					
Fertilized	3	28	36	67	36
Unfertilized	1	16	10	27	
Total ..	15	151	93	259	(100)
Normal Fertilized Ovules ..	67	28	44		36
Seed Set	60	29	53		39
Normal Pollen	44	54	74		61

* Except for total in the last column, figures above the mid-line are frequencies and below the mid-line are percentages.

TABLE IV

Time taken by endosperm to become cellular and fertility data of the clones in the two allopolyploids

Days on which the endosperm became cellular*	Clone No.	Normal fertilized ovules†		% seed set	% sterility
		Number	% of total		
5	7	41	44	53	47
6	2	17	36	31	69
7	1	53	31	20	80
7	4	18	64	48	52
8	3	7	18	24	76
10	6	42	28	29	71

* From Table V.
† From Tables II and III.

TABLE V

Average number of nuclei in the endosperm of E. glaucus *and its amphidiploid hybrids at one day intervals after anthesis*

Days after anthesis	*E. glaucus*	*Glaucus-jubatum*					*Glaucus-hystrix*			
		1	2	3	4	Mean*	5	6	7	Mean*
1	5	5	..	2	2	3	2	..	..	2
2	39	3	28	..	12	14	..	..	19	19
3	226	31	..	..	17	24	..	34	27	31
4	>300 (cellular)	79	53	5	..	45	..	143	111	127
5	..	183	129	..	130	147	149	196	>300 (cellular)	172
6	..	242	>300 (cellular)	113	219	191	..	244	..	244
7	..	>300 (cellular)	..	236	>300 (cellular)	236	..	..	..	..
8	..	..	..	>300 (cellular)	..	>300 (cellular)	..	268	..	268
9	..	..	..	..	..	..	..	..	..	..
10	..	..	..	..	..	..	..	>300 (cellular)	..	>300 (cellular)

* Mean was computed on the basis of the number of embryo-sacs observed in each catogory.

Time taken by the endosperm to become cellular in each clone, appears to bear some relationship to the per cent. of normal fertilized ovules (Table IV)—these ovules are later expected to develop into viable seeds. Cell-wall formation in the endosperm begins at a stage when the nuclei of this tissue have gone through a certain number of divisions. In some clones this stage is reached earlier than in others (Table V), depending on the rate at which those nuclei divide. It is further observed that the sterility is, on the whole, higher in clones in which the endosperm tissue takes longer time to become cellular.

In those ovules in which abortion occurred subsequent to fertilization, abnormalities were first observed in the nucellus and later in the embryo and endosperm. First signs of abortions were observed in the maternal tissue between the second and third day after fertilization. There was deterioration of the cells of the nucellar epidermis which showed dark-stained patches on the external portions of embryo and endosperm (Fig. 6). This dark abnormal maternal tissue was most often observed to be conspicuous near the micropylar and rather than at the chalazal end or the central part of the embryo-sac (Fig. 7). The embryos in the ovules with the abnormal nucellus, did not appear to increase in size at a normal rate but remained small and appeared atrophied (Fig. 8). In most of the cases in which the embryos were subnormal, the number of cells could not be exactly determined since the deeply stained abnormal maternal tissue covered the major part of the embryo.

The deterioration of the endosperm tissue in the abnormal fertilized ovules of the two allopolyploids, was first observed at the time the endosperm was in the multinucleate condition and before it became cellular. These abnormalities in the endosperm were classed into two main categories depending on the condition of the nuclei: (1) those with disturbances in the mitotic behaviour of the nuclei and formation of chromatin bridges and (2) the others that were dark staining and showed signs of deterioration —some of the latter reached such a degree of deterioration that even the nucleoli were not evident (Figs. 9 and 10). In the later stages of development of these abnormal ovules, the abnormal endosperm nuclei did not appear to have cell-walls surrounding them. The antipodals in these abnormal ovules disintegrated at the same time the endosperm became cellular. There were no abnormalities observed in the antipodals; they behaved in the same way as they did in the normal ovules that were fertilized in the clones of the two allopolyploids. The abnormal fertilized ovules

collapsed 10–16 days after fertilization and the tissue of both embryo and endosperm degenerated into an amorphous mass.

The percentages observed in the various categories of normal and abnormal ovules in the two allopolyploids are given in the last columns of the Tables IV and V. The total abortion (the sum of the normal unfertilized ovules and of all the abnormal ovules, both fertilized and unfertilized), was slightly greater in *glaucus-jubatum* (40 + 26 = 66 per cent.) than in *glaucus-hystrix* (28 + 36 = 64 per cent.). On the other hand, *glaucus-hystrix* not only had a higher percentage of normal fertilized ovules (36 per cent.) but it also had a higher percentage of seed set (39 per cent.) and normal pollen (61 per cent.) than that of the *glaucus-jubatum* allopolyploid (normal fertilized ovules 34 per cent., seed set 22 per cent. and normal pollen 39 per cent.). *Glaucus-hystrix* also had a greater variability in seed set among the various clones. Although the number of clones examined in both the allopolyploids was small, the opportunity for selecting lines with a normal seed set seems to be slightly greater in *glaucus-hystrix* than in *glaucus-jubatum*.

Discussion

Seed abortion in hybrids may be considered from two points of view: (*a*) the genetic basis, that is, the underlying genetic mechanism responsible, and (*b*) the abnormalities observed during the development of the mature seed, that is, the specific manner in which the genetic mechanism leading to abortion expresses itself. The great bulk of literature on seed abortion in hybrids has been directed to a study of abnormalities observed in order to understand processes of seed development and to assign specific functions to various structures of the ovule. Relatively few attempts have been made to investigate the genetic basis of seed abortion other than to conclude that it is probably due to inharmonious gene combination—genic sterility. A few cases of inharmonious gene combinations (gene unbalance) have been attributed to unequal distribution of chromosome segments at meiosis-chromosomal sterility.

The F_1 diploid hybrids from both crosses, *E. glaucus* × *S. jubatum* and *E. glaucus* × *S. hystrix*, are completely sterile. On the other hand, when the chromosome number is doubled by colchicine, the C_1 generation obtained is almost completely fertile. The C_2 segregates obtained from seeds produced on the C_1 plants are found to segregate for different levels of fertility. Stebbins and Vaarama (1952, 1954) made a cytological analysis of microsporogenesis in the F_1 hybrids and in the doubled C_1 plants. They came to the conclusion that the sterility in the F_1 hybrids was largely due to the production of spores with duplications and deficiencies at both male

and female sporogenesis. That is, they assumed that the parental species differed from each other by numerous small rearrangements, a condition which they called cryptic structural hybridity. These authors favoured the hypothesis of cryptic structural hybridity largely because there was a restoration of fertility with the doubling of the chromosome number. Since no evidence of gross structural rearrangement was observed in the microspores, they assumed that such rearrangements, if present, would have to be small, and thus not expressed as irregular behavior at meiosis, hence the designation cryptic. The chiasma frequency in the F_1 diploid hybrids was lower than in the parents, which was in keeping with the hypothesis of cryptic structural hybridity.

In the present study seed abortion in the two *Elymus-Sitanion* hybrids has been classified under the following categories: (1) failure of the embryo-sac formation at various stages prior to fertilization, (2) failure of fertilization and (3) ovule abortion subsequent to fertilization.

Failure of the embryo-sac formation.—The evidence obtained in this paper suggests that in the ovules of F_1 diploid hybrids of *E. glaucus* × *S. hystrix*, the deterioration occurs at stages before the embryo-sacs develop to maturity and that the nucellus also degenerates. If the sterility of the F_1 hybrids is due to minute structural differences in the chromosomes between parental species, degeneration of the megaspore-mother-cell during prophase is unexpected, since the postulated duplications and deficiencies should not show their effects until after the first meiotic division. As 41 out of the 44 abnormal ovules examined of the diploid F_1 hybrid appeared to have degenerated at stages following the first meiotic division and before the embryo-sac reached maturity, the embryological evidence would favour the hypothesis that the sterility was manifest chiefly in degeneration of the gametophyte generation as a result of the type of segregation which took place at meiosis. Thus the sterility could be chromosomal rather than genic, in the sense defined above. Nevertheless, genic sterility would offer a better explanation for the abortion of ovules in F_1 plants since the disintegration of maternal tissue (nucellus) seems to play an important role in the deterioration of the ovules in these diploid hybrids. The importance of the maternal tissue in seed sterility will be discussed later.

The overgrowth in one of the three F_1 diploid hybrids may be explained on the basis of interaction of genes, the latter being contributed by both the parental strains. The strain used as a pollen parent was common to all the three of the crosses from which these hybrids were obtained, and the three pistillate plants were from different strains and would, therefore, differ in

their genetic make-up. It is most likely that male and female parents of F_1 hybrid 112 have each contributed one or more factors that interacted to produce the proliferation of cells in the external integument of the ovules in the hybrid. Since such factor or factors were not present in the other two female parents from which hybrids 107 and 111 were derived, these F_1 plants did not exhibit such abnormal development in the ovular tissue.

The breakdown of the ovule at stages before anthesis causing sterility in the *glaucus-hystrix* allopolyploid (Table III, row 4), abnormal unfertilized ovules appeared to be similar to that of the F_1 diploid hybrids and it is, therefore, not unlikely that factors causing breakdown in both the diploid hybrids and in their allopolyploids are the same. Stebbins and Vaarama (1952, 1954) have strongly suggested that the cryptic structural hybridity is largely responsible for seed sterility in F_1 plants. If that is true, then these structural differences could also be responsible for seed abortion in 27 unfertilized abnormal ovules (29 per cent.) of the abnormal ovules in *glaucus-hystrix*. It is not clear why abortion of abnormal ovules before anthesis (29 per cent. or 27 abnormal unfertilized ovules) should occur only in one polyploid (*glaucus-hystrix*) and not in the other (*glaucus-jubatum*). However, it is possible that the structural differences in the chromosomes causing sterility in the former are fewer than in the latter or it could even be that these differences in *glaucus-jubatum* are not sufficiently critical to cause sterility during the stages of embryo-sac formation.

Failure of fertilization.—Unfertilized ovules causing seed abortion have been reported in hybrids from a number of interspecific and intergeneric crosses. Recently Håkansson and Ellerström (1950) have stated that failure of fertilization is more frequent in the tetraploid than in diploid rye. They have also noted that these unfertilized ovules had smaller embryo-sacs with underdeveloped antipodals. Kattermann (1936) reported that in one of the sterile forms of *Hordeum distichum*, 42 per cent. of the unfertilized embryo-sacs had antipodals that were abnormal, although the egg-apparatus and the polar nuclei were normal. As reported earlier in the paper, the apparent causes of ovules remaining unfertilized and ultimately aborting is probably conditioned by their immaturity, as evidenced by the underdeveloped ovules with subnormal antipodals and delayed disappearance of the nucellar tissue.

Ovule abortion subsequent to fertilization.—The hypothesis of abnormal behaviour of antipodals causing irregularities in the endosperm which in turn result in the starvation and death of the embryo was proposed by Brink and Cooper (1944) in respect to the hybrid seed of *Hordeum jubatum*

× *Secale cereale* and supported by Beaudry (1951) with his work on *Elymus virginicus* × *Agropyron repens*. On the other hand, Thompson and Johnston (1945) did not observe any abnormal behaviour in the antipodals and do not agree that the antipodals are the cause of breakdown of the endosperm tissue. Furthermore, the hypothesis of Brink and Cooper could not be used to explain seed sterility in the *Oenothera* type of embryo-sac in which antipodals are not formed. It is not unlikely that the conditions causing abnormalities in the antipodals also cause irregularities in the endosperm and death of the embryo. Observations of the abortive ovules in the two allopolyploids studied here did not show any irregular behaviour of antipodals, except that the deterioration of the antipodals was delayed in the allopolyploids in comparison with the diploid parent.

In a large number of cases dealing with hybrid seed sterility in interspecific and intergeneric crosses in Gramineæ, starvation and death of the embryo is considered to be a consequence of the breakdown in the endosperm. Boyes and Thompson (1937) reported irregularities in the endosperm of the hybrid seed of *Triticum*, Cooper and Brink (1944) in seeds of *Hordeum jubatum* × *Secale cereale*, Håkansson and Ellerström (1950) in rye, Kihara and Nishiyama (1932) in *Avena*, Löwestein (1951) in rye, Thompson and Johnston (1945) in *Hordeum vulgare* × *Secale cereale* and Wakakuwa (1934) in wheat. On the other hand, Sansome *et al.* (1942) on examining the hybrid seed from interspecific cross of *Datura*, observed that in some cases the endosperm disintegrated earlier than the embryo whereas in other cases the embryo disintegrated before the endosperm.

The present investigation shows that mitotic irregularities occur in the endosperm of those seeds in which the first signs of abortion are observed in the maternal tissue (nucellus). In nearly all the cases of seed abortion of hybrids described in the literature, the endosperm irregularities are considered as a major cause of abortion. In the case of the allopolyploids described in this paper, the deterioration of the nucellus seems to be of primary importance in seed sterility. Subsequently this abnormal condition of the maternal tissue is followed by mitotic irregularities and deterioration of the nuclei in the endosperm tissue. The development of the embryo is also retarded. The discussion on the influence of the nucellus in seed abortion will be considered later at greater length.

The explanation given earlier in favour of abnormal behaviour of antipodals and breakdown of endosperm as the main cause of starvation and death of the embryo, are evidently based on the fact that the maternal tissue supplies food elements to the young, growing embryo, through the anti-

podals and the endosperm. Furthermore, the experiments of culturing excised embryos from the hybrid seed of *H. jubatum* × *S. cereale* by Brink *et al.* (1944) have shown the practicability of artificially rearing hybrid zygotes which normally do not reach the mature embryo stage in the seed and that the death of the embryo arises not from the inherent defects in the embryo but is secondary to the changes in the tissues of the ovule. These investigators (Brink *et al.*) have attempted to show that endosperm is the dominant tissue causing breakdown of the hybrid ovule. These investigations of excised embryos, however, do not exclude the possibility of the growing embryo drawing directly upon the food supply in the sporophytic tissue of the female parent. Cooper and Brink (1949) have presented a case in which the embryo of *Taraxacum* is able to absorb nutrients from the integuments of the ovule. The maternal tissue, therefore, may have a direct influence on the developing embryo and endosperm. Consequently, the embryo and endosperm of the aborting ovule, would show abnormalities at different stages of the growth of the seed.

Sansome *et al.* (1942) have observed that in some crosses of *Datura* the embryo begins to disintegrate earlier than the endosperm tissue and in other cases the disintegration of the embryo follows that of the endosperm. Mangelsdorf (1926), on the other hand, has reported certain cases in maize in which genes have parallel effect on the embryo and endosperm. Endosperm, therefore, need not always be a dominant factor in seed sterility. In the class (3) of sterility reported in this paper, the seed aborts after it is fertilized. Abnormalities are first observed in the sporophytic tissue (nucellus) and later in the embryo and endosperm. Apparently, the disintegrated nucellus prevents the passage of the nutrients to the embryo and endosperm, which ultimately causes abortion of the juvenile seed. The deteriorating nucellus is an important factor in seed abortion not only in the two allopolyploids (classes 1 and 3 of seed sterility given elsewhere in this discussion) but also in the F_1 diploid hybrids. As postulated by Stebbins and Vaarama (1954) these abnormal conditions in the maternal tissue were caused by the disharmony of certain gene combinations which otherwise function well to produce normal growth at all times excepting at two stages of development. One critical stage is before the maturity of the embryo-sac and the other is two or three days after fertilization. At these two stages the sporophytic tissue in the ovule is disturbed, which results in abortion of the ovule.

Summary

The sterility in the F_1 diploid hybrids of *E. glaucus* × *S. hystrix* occurred at stages before the embryo-sac reached maturity.

In one of the three F_1 hybrids (112) of *E. glaucus* × *S. hystrix*, the overgrowth of the integumentary tissue was observed. It was postulated that this proliferation of cells was caused by the complementary action of genes contributed by both the parents.

The nature of seed abortion in the two allopolyploids was classified as follows:

(1) Ovules remaining unfertilized which did not seem to be receptive at anthesis.

(2) Deterioration of the nucellus and the structures enclosed in it at stages before the embryo-sac was mature. This sterility was observed only in the *glaucus-hystrix* allopolyploid and was similar to that observed in F_1 diploid hybrid.

(3) Deterioration of the maternal tissue (nucellus) of fertilized ovules followed by mitotic irregularities and deterioration of nuclei in the endosperm tissue and atrophy of the embryo. These abnormalities were found in both *glaucus-jubatum* and *glaucus-hystrix* allopolyploids.

Abnormalities of the maternal tissue were the first signs observed in the aborting ovules in classes (2) and (3) which were, therefore, considered to be the primary cause of seed sterility in the F_1 diploid hybrids and in the two polyploids synthesized from them. Interaction of genes rather than cryptic structural hybridity was suggested as the cause of ovule abortion.

There were two critical stages at which sterility factors became effective. The first was before the embryo-sac reached maturity and the second was two or three days after fertilization.

ACKNOWLEDGEMENT

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EXPLANATION OF TEXT-FIGURES

TEXT-FIGS. 1–10. Fig. 1. *E. glaucus*. A section of the normal embryo-sac at anthesis, ×240. Fig. 2. *E. glaucus* × *S. hystrix*. Megaspore-mother-cell, ×190. Fig. 3. F_1 hybrid 112 (*E. glaucus* × *S. hystrix*). Longitudinal section of the deteriorating ovule before anthesis, ×240. Figs. 4 and 5. F_1 hybrid 112 (*E. glaucus* × *S. hystrix*). Longitudinal sections of the two ovaries at stages before anthesis showing complete degeneration of the nucellus, ×110. Fig. 6. *Glaucus-jubatum* allopolyploid. Section of the ovule with patches of the deteriorating nucellus six days after anthesis, ×280. Fig. 7. *Glaucus-hystrix* allopolyploid. Same as in Fig. 6, four days after anthesis, ×250. Fig. 8. *Glaucus-hystrix* allopolyploid. Section of the ovule collected eight days afer anthesis, showing an embryo that appears to have a retarded development, ×250. Fig. 9. *Glaucus-hystrix* allopolyploid. A section through the endosperm six days after anthesis, ×330. Fig. 10. *Glaucus-jubatum* allopolyploid. Same as in Fig. 9, × 330.

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FOSSIL FORAMINIFERA FROM THE CRETACEOUS ROCKS OF SOUTH INDIA

Part I. Ariyalur Area Orbitoids

By L. Rama Rao, F.A.Sc.

Received March 30, 1957

1. General Introduction

It was clear from the reports of the pioneer geologists,—Blanford (1865), King (1880), and Kossmat (1897) that we have along the east coast of South India several small but nevertheless very interesting exposures of marine fossiliferous Cretaceous rocks, due to a transgression of the southern sea in this region during the upper Cretaceous period. During the last thirty years, the present writer has been engaged in the study of the stratigraphy and palaeontology of these rocks, and a number of papers dealing with several aspects of these researches have been published from time to time. A connected account embodying the results of these contributions has been just published by him in the *Proceedings of the Academy*;[10] a reference to this paper would be helpful in furnishing the reader with the necessary full background for appreciating the geological setting of the several rock formations referred to in the present foraminiferal studies and their special importance.

Very early in the course of this work, it occurred to the present writer that one of the most valuable and interesting lines of investigation would be the study of the Foraminifers from these rock formations about which only a passing reference had been made now and then by the pioneer workers. In view of the present recognised importance of this group of fossils in the study of Cretaceous rocks all over the world, it was obvious that similar work in the rocks of South India was urgently called for. Starting on this line of enquiry nearly thirty years ago, the author recorded in the year 1924 the occurrence of numerous small Foraminifers in the phosphatic nodules of the Utatur group; and this was followed by the find of similar microforaminifers in various other beds of the South Indian Cretaceous by a number of workers,—the genera commonly noticed as a result of these studies being *Nodosaria*, *Textularia*, *Planorbulina*, *Anomalina*, *Rotalia*, *Robulus*, *Lenticulina*, *Nonion* and *Globotruncana*. Special attention was also drawn by the writer to the occurrence of abundant Miliolidae in some of the limestones

of the Niniyur group. Recent investigations have further revealed the presence of several other important groups of Foraminifers in these Cretaceous formations the study of which has now been undertaken by the author with reference to certain beds in the Trichinopoly and Pondicherry areas; the present paper deals with a general account of the Orbitoidal fauna recently noticed in some of the rocks belonging to the Ariyalur group.

I am much obliged to several of my erstwhile colleagues in the Central College for all the assistance they have given in the preparation of the material and taking the photomicrographs used in illustrating these papers. I would also like to thank Sri. Y. Nagappa, Palaeontologist, Assam Oil Co., Ltd., for helpful discussions.

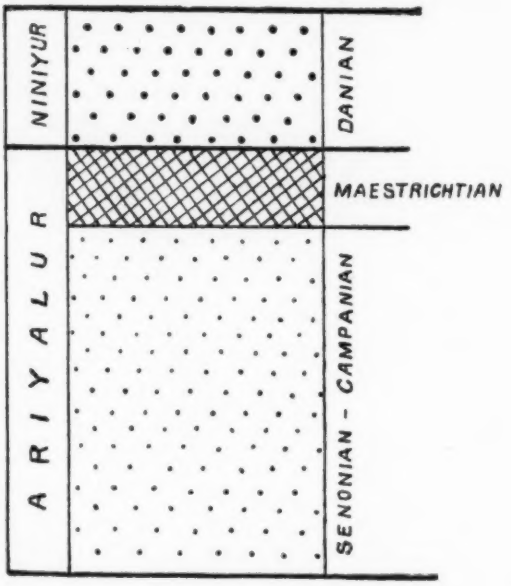
2. STRATIGRAPHY OF THE AREA

Before proceeding to deal with the Foraminifers, it will be useful to recapitulate briefly the stratigraphical classification of the Cretaceous rocks of the Trichinopoly District (which is the type area for these coastal Cretaceous rocks) and indicate the position therein of the beds from which the Orbitoids are now being described. The Trichinopoly Cretaceous is divided into 4 groups; starting from the oldest these are: (i) the Utatur, (ii) the Garudamangalam (Trichinopoly), (iii) the Ariyalur, and (iv) the Niniyur. The entire succession ranges in age from the upper Albian to the Danian of the European stratigraphical scale.

The Foraminifera now under study all come from a collection of certain arenaceous limestones occurring as bands a few miles to the east of Ariyalur; stratigraphically their position is just below the Danian Niniyur group, and representing the topmost members of the Ariyalur division. Their age would thus be Maestrichtian. The exact stratigraphical position of these beds in the Ariyalur-Niniyur succession is shown in the diagrammatic section given below (*vide* Text-Fig. 1).

3. PREVIOUS WORK

The earliest reference to the occurrence of Foraminifers in this area was by Stoliczka in 1873[12] who found them in some of the limestones collected by H. F. Blanford from Niniyur and Chokanadapuram about 10 miles NNE of Ariyalur. In this collection, Stoliczka noticed the occurrence of some Orbitoids represented by " a single well defined species, *Orbitoides faujasi* Defr. and two doubtful ones". The *O. faujasi* of Stoliczka and its subsequent nomenclatural history has been recently reviewed by the author elsewhere,[9] and it is now clear that this form is the same as what is now called *Lepidorbitoides minor*. More recently, after nearly seventy years after Stoliczka's



TEXT-FIG. 1. Diagrammatic section of the Ariyalur-Niniyur succession showing the position of the Maestrichtian band of limestone containing Orbitoids and Siderolites.

work referred to above, S. R. Narayana Rao was able to collect more of these Orbitoids from near Chokanadapuram, and he published a short account of these in 1941.[5] He has recognised here three species (all new) of Orbitoids,—one of *Orbitocyclina* (*O. ariyalurensis*), and two of *Lepidorbitoides* (*L. inornata* and *L. blanfordi*). According to this author, the *O. faujasi* identified by Stoliczka in 1873 is a *Lepidorbitoides* belonging to his new species *L. blanfordi*.

It may in passing be pointed out that while describing *O. faujasi*, Stoliczka had mentioned that the shell varies from 4 to 8 mm. in diameter; but later both Vredenburg[14] who was redescribing this material and S. R. N. Rao who was dealing with the Orbitoids from the same area, recorded that none of the shells in their collection were more than 4 mm. in size. Some years ago, the present writer collected a few specimens of an orbitoidal limestone near Coothoor (about 4 miles south of Chokanadapuram) and it was interesting to find that the shells here were much bigger (8–9 mm. in diameter) in size; the bigger forms referred to by Stoliczka and subsequently lost sight of were probably similar to the ones collected from near Coothoor. But unfortunately, no further work was done on these Coothoor

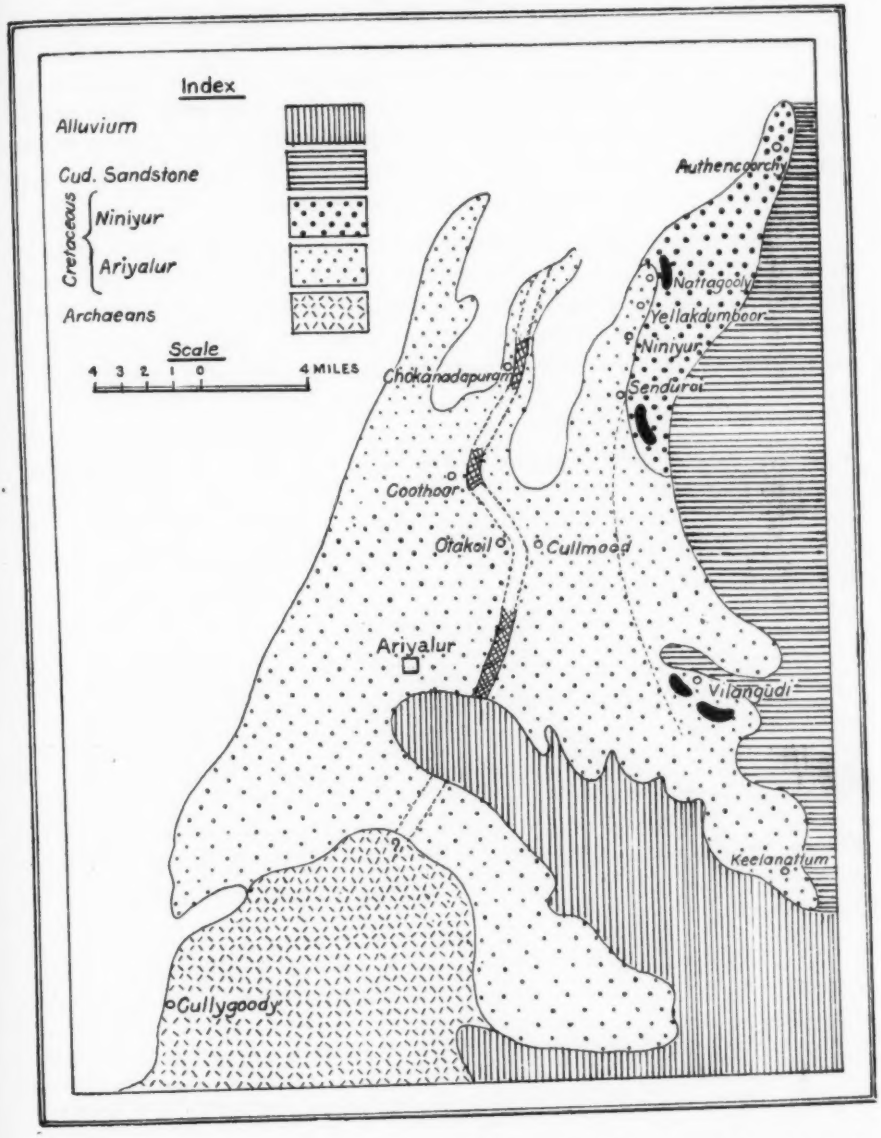
Orbitoids; this area evidently needs further exploration. It is also specially interesting to note that in one of the limestones occurring in a locality so far south as Cullygoody (Kallakudy), Thalmann is recently reported[4] to have found some *Lepidorbitoides*,—an occurrence altogether unexpected according to our current notions regarding the age of the rocks in that region (Cenomanian).

4. PRESENT WORK

The above is a brief review of our knowledge till recently of the Orbitoids from the Trichinopoly Cretaceous beds; and it will be readily admitted that this record is by no means satisfactory or impressive. Such a review however reveals to us the vast scope for foraminiferal research in this area, and points out the need for more extensive studies in this fascinating field.

About two years back, when examining sections of some of the rocks from the Ariyalur area (collected by a geological excursion party from the Central College) the present writer found that some of the rock sections revealed the presence of numerous Orbitoids;[6,7] in addition they also contained abundant and well preserved *Siderolites*.[8] Numerous small Foraminifers also occur quite commonly belonging to the genera ***Rotalia***, ***Lenticulina***, ***Operculina***, ***Anomalina***, ***Amphistegina***, ***Cibicides***, and ***Camerina*** (?). In view of the importance of this find and the very promising nature of the material, a larger and more representative collection of some of these selected rocks was later obtained for detailed study through the kindness of Sri. S. Subramanian, Geologist of the F.A.C.T. (Ltd.) in Ariyalur. From the general trend and disposition of these rocks in the field, it would appear that the Orbitoidal beds now noticed east of Ariyalur are the southern extension of the similar Orbitoidal beds already noticed near Coothoor and Chokanadapuram, and probably represent portions of one and the same band as indicated in the map given below of the Ariyalur area (Text-Fig. 2). It would be most fruitful to survey the area all along this line, and also to look for possible continuations of this band north of Chokanadapuram on one side and south-east of Ariyalur on the other. It seems likely that the limestone near Cullygoody (Kallakudy) in which Thalmann is reported to have found *Lepidorbitoides* (referred to above) is probably a representative of this band at its southern extremity (*vide* Text-Fig. 2).

The rock specimens from near Ariyalur now under study were collected from two adjacent bands of limestones, one of them crowded with Gryphea shells, and the other full of Inoceramus shell fragments. The latter (Inoceramus bed) occurs just east of the former (Gryphea bed), both of them with a general north-south strike. Although the two rocks look similar and are



TEXT-FIG. 2. Map of the Ariyalur area showing the Maestrichtian limestone band (containing Orbitoids and Siderolites) occurring to the east of Chokanadapuram, Coothoor, and Ariyalur, and its probable full extent.

found close together, there is a striking difference in their foraminiferal contents. While some of the smaller Foraminifers are no doubt common to both, a significant fact is that all the Orbitoids and Siderolites which are so abundant are exclusively confined to the Gryphea bed and totally absent from the adjacent Inoceramus bed. There appears to be thus a sharp micro-palaeontological boundary between the two, and the exact meaning of this is not clear. This is a point requiring further attention.

The present paper deals only with the Orbitoids noticed in these Ariyalur rocks, and is based on the study of about 25 rock specimens of which nearly 100 sections have been prepared and examined. It must, however, be pointed out that most of the observations recorded here are based on the study of rock slides wherein we get only sectional views of these Foraminifers often in random directions; at times, though rarely, these shell sections in some of the slides are recognisably 'oriented' and these are the ones that have been selected for description here. For fuller and more conclusive studies, it is necessary to separate the shells from the rocks in which they are found and base all our identifications on such prepared material. Luckily most of the Ariyalur rocks are well suited for such treatment, and work in this direction is under progress.

5. DESCRIPTION

By far the most common among the Foraminifers occurring in the present material (confined however to the Gryphea bed) are the Orbitoids,—some of the slides being crowded with their remains (Plate XXVIII, Fig. 5). Most of these are random sections and often fragmentary; but now and again we get some nice vertical or transverse sections quite useful for study. A few good equatorial sections, though often only partial, are also noticed.* All the sections show the usual Orbitoidal characters and the photomicrographs reproduced in the accompanying Plates give a good general idea of these forms.

One striking feature is that these shells as a whole are small in size, much smaller than the generically similar forms described from other Cretaceous

* In naming the orientation of these sections, the following is the terminology used by the author: sections in the median plane including the entire circumference and passing right through the centre are designated 'equatorial' sections; sections at right angles to the median plane and passing through the centre are called 'vertical' sections. Sections in a plane parallel to the equatorial but not cutting the centre are named 'horizontal' sections, while sections parallel to the vertical, but not passing through the centre will be referred to as 'transverse'. All other sections not conforming to any of the above recognisable orientations will be generally described as 'oblique'.

beds from India or outside.† Many of these are about 2 mm. or less in diameter; occasionally we get some whose diameter is about 3 to 4 mm. The thickness and its ratio to the diameter are also quite variable,—some being short and stout, and others thin and flat. The following measurements made on some vertical sections will give an idea of the variation in size and proportion:

Diameter	Thickness
(in mm.)	
3·0	0·75
2·3	0·53
2·0	0·8
1·13	0·33
0·86	0·23
1·4	0·3
1·16	0·46
1·93	0·53
1·33	0·43

Several of the sections belong to the megalospheric forms and show a bilocular nucleoconch (of which the first is almost spherical, and the second, slightly bigger in size, is more or less reniform in shape partly embracing the first) similar to that of *Lepidorbitoides*; the shape and arrangement of the equatorial chambers are also generally similar. The existence of stolon passages connecting the adjacent equatorial chambers is often clearly seen; so also the 'cribriform perforate' nature of the chamber walls. Pillars of varying prominence are also commonly noticed.‡ From all these characters, it is seen that many of these shells belong to the genus *Lepidorbitoides*. The question whether there is no true *Orbitoides* in this area had better be

† Quite recently, however, some Orbitoids of a similar small size have been described by H. H. Renz [*Micropalæontologist*, Jan. 1955, **1** (1)] from the upper Cretaceous (Maestrichtian) of North-Central Venezuela.

‡ In describing certain Lepidocyclinas from the Bikini Drill Holes, Storrs Cole (U.S.G.S. Professional Paper 260–O, 1954) has pointed out that in some of these, we get structures which look like 'pillars' but are really not so; *e.g.*, in his description of the form *L.* (*Eulepidina*) *gibbosa* he says: "The roofs and floors of the lateral chambers are thin at the centre, but thicken at each end. The thickened ends of the floors and roofs of one tier of chambers join those of the next adjacent tier on each side to produce a column-like structure between the tiers of chambers. However, pillars are not present." Then again, in *L.* (*Eulepidina*) *planata*: "The lateral chambers are arranged in definite tiers. The rectangular chamber cavity is open, and bounded by relatively heavy floors and roofs. The line of juncture between the tiers of lateral chambers is thickened giving the appearance of pillars. However, true pillars are not present."

Some of the Orbitoids in the present Ariyalur material also show a similar feature.

left open pending examination of a much larger number of properly centred equatorial sections showing details of the embryonic apparatus. After giving this general account of the Orbitoids seen in the present material, we now proceed to give a detailed description of some of the forms with special reference to their interesting features; these have been illustrated in the accompanying photomicrographs.

Plate XXVII, Fig. 1 (×22) shows all the usual features of an Orbitoid seen in vertical section; but unfortunately the nucleoconch region is not well preserved. The form is lenticular (but slightly asymmetrical), sloping gradually on either side towards the periphery. Its diameter is 3 mm. and thickness 0·75 mm. The equatorial zone is not very prominent; its chambers are gradually increasing in size, their height and width however remaining almost equal. The lateral chambers are clearly arranged in regular tiers of which there are 12 in the centre and only 5 or 6 towards the end; the lateral chambers are flat, the length as measured in the central region is about 66–80 μ, and the height 16–24 μ. The bounding walls are about 20–25 μ in thickness, which is more or less equal to the height of the chambers. Under high magnification, fine stoloniferous passages are also seen connecting a given lateral chamber with the one immediately below and the other immediately above it in the adjacent tiers. Small 'pillar-like' structures between the adjacent rows are seen, but these seem to be merely due to the fusion of the lateral walls of the adjacent chambers; there are no true pillars as such, and there is no indication of any surface ornamentation.

In many respects this form is similar to *Lepidorbitoides inornata* already described from this area. S. R. N. Rao who first described this species[5] says that externally this test is indistinguishable from his *Orbitocyclina ariyalurensis*; the difference between the two however becoming evident in the nature of the embryonic apparatus as seen in equatorial sections. *L. inornata* is also said to resemble very closely *L. rutteni*, Thiadens described from Cuba;[13] but according to Thiadens, *L. rutteni* also possesses a periembryonic spiral of 6–10 chambers following the bilocular nucleoconch. In some of its characters the present Ariyalur form also resembles *L. palmeri* Thiadens from Cuba; but there again, we have the spiral of periembryonic chambers after the initial chambers. In view of the fact that a spiral of periembryonic chambers is a distinctive feature common to all these forms which otherwise no doubt resemble *Lepidorbitoides*, the question arises whether they should all be considered as species of *Lepidorbitoides* itself, or should be taken out from there and placed in a new genus *Orbitocyclina*. This has been briefly discussed in the later part of this paper.

Plate XXVII, Fig. 2 ($\times$ 35) is the transverse section of an Orbitoid, where the shell appears to be flat, rather curved—slightly asymmetrical and gradually tapering towards the peripheral margin. The length of the form is 2·3 mm. and the width 0·5 mm. Since the section is not truly vertical these measurements do not represent the diameter and thickness of the entire shell though they would give us some idea of the proportion between the two. The equatorial zone of chambers is quite clear; these chambers are squarish in outline with curved outer walls and slightly equitant. The presence of a median stolon connecting the adjacent chambers is clearly indicated all along the equatorial zone. The number of tiers of lateral chambers on each side is small—7 in the centre and only 2 or 3 at either end. The lateral chambers are flat, their length being about 3 or 4 times the height. The most triking feature of this form is the presence of pillars arranged more or less at regular intervals on either side all along the median zone, and appearing as knobs on the surface. The pillars are bigger in the centre and get gradually reduced in size as we proceed towards either end. The surface diameter of the central pillar (seen clearly in the photograph) is about 66 μ.

The Orbitoid in Plate XXVII, Fig. 3 ($\times$ 40) is a very interesting form,—the pillars being its most striking feature. The section is almost certainly vertical; the region of the nucleoconch as also parts of the equatorial zone have however been badly damaged during fossilization. The form is stout in the centre and rapidly tapers towards either end in a characteristic manner. The diameter is about 2 mm., the thickness in the centre is 0·8 mm., while towards either end it gets reduced to 0·3 mm. The form is asymmetrical, and there is a corresponding variation in the number of tiers of lateral chambers on either side; for instance, in the central region we have 12 of these on one side, and only 7 on the other. The walls of the lateral chambers are thick. Under high power we see the fine stoloniferous passages connecting the lateral chambers as in the form in Fig. 1. The shell possesses well developed pillars on either side along the median zone, the 3 in the centre which seem to radiate on either side from the region of the nucleoconch being much more prominent and ending in distinct knobs on the surface, their surface diameter being about 100 μ. It is interesting to note that the central pillar on one side seems to get forked with the result that we see 4 surface knobs on that side as against 3 on the other. As we go away from the centre towards either end, the pillars get reduced in thickness; one of them about half-way down has a terminal thickness of about 66 μ.

In the general shape of the shell and the possession of particularly well developed pillars especially in the centre of the test, this form is similar to

the new species *Lepidorbitoides blanfordi* of S. R. N. Rao.[5] According to him, this species is quite distinct from the typical *L. minor* of Maestricht, and includes in its synonymy Vredenburg's *Orbitoides minor* (= *O. faujasi*, Stoliczka) from this very area.

Plate XXVII, Fig. 4 (× 70) shows the vertical section of an interesting form, very nicely preserved. Its diameter is 1·16 mm. and thickness 0·46 mm. The shell is much inflated in the centre and slopes fairly rapidly towards either end where the thickness is only about 0·12 mm. The median zone of equatorial chambers, as also the tiers of lateral chambers, are well seen. The equatorial chambers are clearly equitant and show the usual arched roofs. There are 7 tiers of lateral chambers in the centre and these get reduced to 3 towards the outer margin. In size, the lateral chambers are about 66 μ long and 16 μ high. The chamber walls are thick (12–14 μ), and almost equal to the height of the chambers. Under high magnification, the fine stoloniferous passages connecting the proximate lateral chambers in adjacent tiers are very clearly seen. The embryonic apparatus as seen in this section indicates a bilocular nucleoconch which is very distinctive; the smaller locule is triangular and the bigger one, spherical; the base of the former measuring 50 μ is just in tangential contact with the latter which has a diameter of 83 μ. The nucleoconch is enveloped by a well defined common wall measuring about 16 μ in thickness. The most striking feature of the shell is the prominent and very stout central pillar on one side, ending in a conspicuous knob on the surface. Its thickness at the inner end is 100 μ, and this rapidly increases to 166 μ at the outer end. Otherwise there are no pillars on either side, and the surface of the shell is quite unornamented.

This form has some general resemblance in its characters to a type of *L. blanfordi* figured by S. R. N. Rao[5] (Pl. VI, Fig. 8 of this paper) and may perhaps belong to the same species.

Plate XXVIII, Fig. 6 (×45). The shell in this case is clearly asymmetrical, with the nucleoconch also eccentrically placed, being shifted towards the more convex side of the shell. The diameter of the form is 1·93 mm. The unequal development of the lateral chambers on either side of the median zone is clearly seen, the thickness on one side being 0·2 mm. and on the other, 0·33 mm. thus making up a total thickness of 0·53 mm. There are numerous pillars arranged all along starting from the equatorial zone and terminating on the surface in knobs; they are of uniform size with a terminal thickness of about 50 μ. The details regarding the equatorial and lateral chambers are not clearly preserved.

The nucleoconch, however, is well seen. It is bilocular and enveloped by a distinct common wall. The first chamber is spherical with a diameter of 83 μ while the second one is bigger in size (Diam. 116 μ) and reniform in shape embracing nearly half of the earlier chamber.

Plate XXVIII, Fig. 7 ($\times$ 25). In this photograph we see a long and thin section of an Orbitoid, the length being 4·4 mm. and the width only 0·33 mm. The median zone of chambers, however, is quite prominent with a width of 83 μ. There are only 3 or 4 tiers of lateral chambers on either side and the total thickness of these on each side is only about 0·12 mm. Judged by these features what we see here may be a transverse section of a thin flat form like *L. rutteni* of Thiadens; or else it may represent a transverse section cut far away from the centre of a microspheric form with a diameter much more than 4·4 mm.

Plate XXVIII, Fig. 8 ($\times$ 75). We have here an Orbitoid of special interest. It is a transverse section of a shell obviously flat and discoidal, with practically no great variation in thickness from end to end. Its length is 0·95 mm. and width, 0·25 mm. The median zone of chambers is quite clear, but the lateral zones are very indistinct with little or no indication of the 'lateral chambers in tiers' seen in the usual Orbitoids. There are no pillars. The most interesting feature shown by the form is that the equatorial chambers in the median zone show in several places a wavy and 'alternating biserial' arrangement with clear indications of connecting stolon passages. The shell probably belongs to the genus *Monolepidorbis*, which according to Galloway is a true Orbitoid, but with lateral zones of the nature of laminae. According to Cushman the genus *Monolepidorbis* "needs further study to place it accurately". The present form, which is the only one of its kind noticed in the Ariyalur material, is evidently of special interest.

Plate XXIX, Fig. 9 ($\times$ 75). We have here a vertical section of a shell with several unusual features. The form is lenticular, inflated in the centre and rapidly tapering towards either end thus resulting in a distinct rim. The diameter of the shell is 1 mm. and its thickness in the centre is 0·42 mm. The form is distinctly asymmetrical with a smoothly curved outline on one side and a prominent triangular and pointed umbonal elevation on the other. The shell is therefore of unequal thickness on either side, being 0·16 mm. in one case and 0·26 in the other. Its most distinctive feature is the presence of a prominent spiral of fairly big sized chambers on one side along the median line. This starts with a bilocular embryonic apparatus of which the bigger spherical locule is about 100 μ in diameter, followed by a series of 5 to 6 fairly big sized and flattened chambers (diameter measuring about 116–132 μ)

arranged in a spiral and possessing distinct chamber walls. The later chambers of this spiral appear to be split up along the median line, accompanied by an 'alternating' arrangement of the divisions on either side. The presence of stoloniferous passages is also indicated especially in the region of the earlier chambers. A careful consideration of the above features makes it doubtful whether the form is really an Orbitoid; on the other hand, one sees here a striking resemblance to certain transverse sections of *Helicostegina* (*e.g.*, *H. dimorpha*) described by Barker and Grimsdale.[2] This is obviously an interesting form, but its exact identity must be considered as still uncertain.

Plate XXIX, Fig. 10 (× 65). Here we have a nice equatorial section (but not complete) of an Orbitoid showing a bilocular nucleoconch surrounded by a spiral of periembryonic chambers, generally similar to what has already been noticed by S. R. N. Rao in his *Orbitocyclina* (*O. ariyalurensis*) from this very area.[5] The two chambers of the nucleoconch are almost equal in size, one being spherical with a diameter of 83 μ, and the other slightly abutting against the first with a diameter of 66–70 μ. The nucleoconch is followed by a distinct spiral of periembryonic chambers of which 11 could be counted (probably there are more); of these, the first 5 gradually increase in size (diameter of chamber 5 = 66 μ) and form a girdle in contact with the outer wall of the nucleoconch, and the following 6 mark the extension of the spiral but quite away from the centre. Both the chambers of the nucleoconch as well as the succeeding periembryonic chambers (especially the first 5) show thin but distinct chamber walls enclosing each of them. These walls which are of clear transparent material are sharply defined since the chamber cavities themselves are infilled by dark ferruginous matter. Some of the later chambers of the spiral become helmet-shaped and show what look like median stolons passing from one to the other.

Outside the spiral, the section shows very clearly, in places, the nature and arrangement of the equatorial chambers. These show the typical arcuate shape with curved outer margins and pointed inwards with slightly incurved sides. The walls of the chambers are thick and juxtaposed. The chambers are alternating in adjacent rows and are so arranged that "the outer walls of the chambers in one circle form the inner walls of the chambers in the following circle".

The nature of the nucleoconch and the shape and arrangement of the equatorial chambers would *prima facie* suggest the reference of this form to the genus *Lepidorbitoides*; but the occurrence of a spiral of periembryonic chambers (also noticed in 3 or 4 other sections in the present material) makes the position rather interesting and raises certain questions regarding their

generic assignment. As already mentioned above, this will be referred to and discussed in a later part of this paper.

Plate XXIX, Fig. 11 (× 45). One of the slides contains an almost complete equatorial section, only a part of which (in the central region) is seen reproduced in this picture. The full diameter is about 5 mm. There is no prominent bilocular embryonic apparatus in the centre; on the other hand, we see even there whorls of small chambers. This suggests that the section is probably of a 'microspheric form'. As we proceed away from the centre, the equatorial chambers gradually increase in size (from 33 μ to 66 μ) and become quite distinct. The chamber walls are quite thick (33 μ in the outer regions) and the outline of the chambers as determined by the chamber walls becomes ogival, though the chamber cavities themselves are rounded in shape. The chambers are alternating in adjacent circles. Under high power, the 'stolon system' is clearly seen, there being as many as 5 or 6 stolon passages radiating from each chamber and connecting it with the adjacent ones.

The general form and arrangement of the equatorial chambers noticed here are similar to what has been described by Thiadens[13] in two of his new species of *Lepidorbitoides* (both from the upper Cretaceous of Cuba)—*L. rutteni* and *L. palmeri*. It may however be noted that in both these species, Thiadens has recorded the presence of a 'spiral' of periembryonic chambers surrounding a typical *Lepidorbitoides*-type of bilocular nucleoconch.

Plate XXIX, Fig. 12 (× 100). This figure again shows part of an equatorial section; but the chambers here are distinctly rhombic or diamond-shaped in outline, the longer diagonal measuring 83 μ and the shorter 50 μ. Towards the peripheral margin the chambers become flattened and their size also gets reduced to 58 $\mu \times 33\ \mu$. The chambers are arranged in intersecting arcs and are alternating in position in adjacent circles. The chamber walls are thick and prominent (16–20 μ), but there are distinct stolon passages connecting them with one another.

While it is true that a rhombic outline is one of the several possible shapes of the equatorial chambers in Orbitoids, it has been recognised as a significant feature of certain species. Vredenburg[14] noticed 'rhombic' equatorial chambers in 3 of the species of *Orbitoides* described by him, *viz.*, *O. media*, *O. apiculata*, and *O. minor*,—the last one being the same as *O. faujasi* described by Stoliczka from the present area. The disposition of the equatorial chambers in the present case shows a striking similarity with that in *O. cf. minor* figured by Vredenburg. More recently, in his description of a new species of *Lepidorbitoides—L. macgillavryi*, Thiadens

says that in this form the embryonic apparatus is of the *Lepidorbitoides*-type, and the equatorial chambers are " ogival to diamond-shaped on intersecting curves, the diagonal measuring 74 μ near the periphery". He considers the possession of ' diamond-shaped ' equatorial chambers as a very distinctive feature of this species.

6. REVIEW AND DISCUSSION

The above is a brief account of some of the Orbitoids noticed in the rocks from near Ariyalur. In addition to these many of the slides contain numerous other Orbitoidal remains; several of them are incomplete or fragmentary sections, but are nevertheless useful in showing one or more of the characters noticed in the forms described above and therefore useful in confirming our observations. Generally speaking, it may be mentioned that the Orbitoids in the present material are much more varied and abundant than in any of the previous collections from this area; the author, however, would like to make it clear that in describing the forms individually and separately as is done above, no suggestion is intended to be conveyed that each of them is a new type,—generically or specifically,—and distinct from the others. It is quite possible that some of these shells are referable to forms previously described from this area or outside, while others are new; but the writer has hesitated to definitely designate them as such since he is of opinion that it is desirable to await the results of further investigations before finalising such conclusions. It is well to exercise a certain amount of restraint in all palaeontological work, especially in creating new genera and/or species; this is particularly necessary in dealing with a group like the present one (Orbitoididae) where it is well known that " even individuals within a single species vary tremendously ". The present studies, however, are enough to bring out certain important points which we may now proceed to indicate and discuss.

The first question which we may consider at the outset is regarding the occurrence of *Lepidorbitoides minor* in these Ariyalur rocks. Our starting point in this discussion is the first Orbitoid,—*O. faujasi*,—described by Stoliczka from this area so far back as 1873. This was later re-examined by Vredenburg; he noticed that in its characters this form agreed so well with that of *O. minor* Schl. of Maestricht that he accordingly renamed Stoliczka's orbitoid and identified it with *O. minor*. In 1907, Silvestri created the genus *Lepidorbitoides*; and in 1916, Douville showed that Vredenburg's *O. minor* really belonged to the genus *Lepidorbitoides* with the result that the old *O. minor* of Vredenburg would now be called *L. minor*. In his recent paper on the Ariyalur Orbitoids, S. R. N. Rao says that the forms here differ

in several respects from the typical *L. minor* of Maestricht, and should therefore be considered as belonging to a new species which he called *L. blanfordi*; in the synonymy of his new species he includes *O. faujasi* of Stoliczka and *O. minor* of Vredenburg. Thus according to S. R. N. Rao, it would appear that there is no true *L. minor* in the Ariyalur beds.

It is hardly possible at this stage to go into the details and discuss all the arguments in favour of or against the above view. The characters fixing the identity of the typical *L. minor* of Maestricht have been recently given by Thiadens,[13] and it appears to the present writer that there are several Orbitoidal sections in the Ariyalur rocks which show features (in the shape of the equatorial chambers, nature of the pillars, etc.) considered as diagnostically characteristic of the type *L. minor* as defined by Thiadens. Even granting that we have here a new species of *Lepidorbitoides*—*L. blanfordi*—as made out by S. R. N. Rao, the fact remains that shells answering to the description of true *L. minor* also occur in these rocks side by side. On the evidence of the present material, we may reasonably conclude that *L. minor* does occur in the Ariyalur rocks.

The next question which we may proceed to briefly consider is regarding the significance of the occurrence of a whorl (or spiral) of periembryonic chambers noticed in some of these Orbitoids as mentioned in the descriptive notes given above. Vaughan considered that this feature was of generic value and therefore created in 1929 the new genus *Orbitocyclina* to cover this type; and it is following this view that S. R. N. Rao recorded the occurrence of *Orbitocyclina* (*O. ariyalurensis*) from the Ariyalur beds. The genus *Orbitocyclina* resembles *Lepidorbitoides* in all respects, its only distinctive character being the possession of a cycle of periembryonic chambers. Within recent years more or less similar periembryonic chambers have been noticed in many species of *Lepidorbitoides*, and the question has been raised whether this character is of such great significance as to warrant the removal of these forms from the genus *Lepidorbitoides*, and creating a new genus *Orbitocyclina* to accommodate them, especially after M. G. Rutten[11] has shown that the stoloniferous apertures also in both the genera are similar; in fact, Rutten questioned the validity of the new genus *Orbitocyclina* of Vaughan and actually stated that it was only a synonym of *Lepidorbitoides*. A couple of years later Thiadens[13] noticed the occurrence of a periembryonic spiral of chambers in his 3 new species of *Lepidorbitoides* from the upper Cretaceous of Cuba, *L. rutteni*, *L. palmeri*, and *L. magillavryi*,—and affirmed that the synonymy of the two genera—*Lepidorbitoides* and *Orbitocyclina*—is "definitely proved". It is not clear, however, whether the exact arrange-

ment of the periembryonic chambers noticed by Thiadens in his 3 species of *Lepidorbitoides* is similar to that seen in the Ariyalur Orbitoids referred to above; judged by the figures given by him, it would appear that there is a distinct difference.

Apart from the synonymy of these two genera, the more interesting and important question to consider is the evolutionary significance of the occurrence of such a periembryonic spiral of chambers. In this connection reference has to be made to 3 other genera,—*Helicolepidina* (Tobler, 1922), *Polylepidina* (Vaughan, 1929) and *Actinosiphon* (Vaughan, 1929)—which show a comparable feature. In the words of Galloway, we have in *Helicolepidina*: " megalospheric nucleoconch bilocular, composed of a spherical chamber followed by a smaller hemispherical chamber, and these followed by smaller arcuate chambers arranged in a spiral." In *Polylepidina*, as Cushman puts it, we have 4–10 embryonic chambers " of which one or two may be somewhat larger than the others..... The chambers may be arranged also in a distinct spiral". In *Actinosiphon*, the position is a little different. Here " the embryonic apparatus of the megalospheric form consists of a rather large subspherical initial chamber, followed by a smaller chamber whose longer diameter is parallel to the circumference of the first chamber. The latter chamber is followed by about 11 other chambers which entirely encircle the first two chambers, and outside this circle, there are several other chambers opposite chamber No. 3 " (Cushman).

It will thus be seen that there are 4 genera in the Orbitoididae possessing distinct periembryonic chambers round the nucleoconch: (i) *Lepidorbitoides* (certain species), (ii) *Helicolepidina*, (iii) *Polylepidina*, and (iv) *Actinosiphon*; and the problem is to discuss the significance of this feature and its evolutionary importance. A perusal of the recent literature on this subject shows that there is considerable difference of opinion, and it is hardly possible to go into the details of this discussion here.

A brief reference may however be made to the problem regarding the genus *Orbitocyclina*—whether it should be accepted as a distinct genus as proposed by Vaughan in 1929, or whether it should be treated merely as a synonym of *Lepidorbitoides* (or alternatively as its sub-genus) as argued by later workers. While it is true that *Orbitocyclina* resembles *Lepidorbitoides* in several respects, such as the form of the nucleoconch, shape of the equatorial chambers, and the nature of the stoloniferous passages, there seems to be a clear difference in the arrangement of the periembryonic chambers between the two genera. The kind of spiral arrangement, its nature and disposition noticed in the *Orbitocyclina* described by S. R. N. Rao, and also

seen in the form described above (Pl. XXIX, Fig. 10) is something distinctive and has no resemblance to what is seen in some *Lepidorbitoides*; on the other hand, it is essentially similar in the present case to the 'spiral' found in *Helicolepidina*. Paying due regard to this important fact, and remembering its possible implication in evolutionary studies, it would appear that it is desirable to retain Vaughan's *Orbitocyclina* as a distinct genus and accept its validity. If there are any *Lepidorbitoides* which show this particular type of spiral of periembryonic chambers, they may accordingly be transferred to *Orbitocyclina*. If this is done, *Orbitocyclina* would have a definite position in the evolutionary series; and Barker's suggestion[1] in connection with *Helicolepidina* "that a possible lineage may be found through *Polylepidina* to some form such as *Orbitocyclina*, the spirality observed in these genera reaching a maximum in *Helicolepidina*" would become more meaningful. In a later paper, Barker and Grimsdale[2] have made out a case to show that *Polylepidina* and *Helicolepidina* are "descended from *Amphistegina* through the new genera *Helicostegina* and *Eulinderina* by a complete series of transitional forms". According to these authors, an Amphistegina-like ancestor gave rise ultimately to *Helicolepidina*, the intermediate links in the chain being *Helicostegina*, *Eulinderina*, and *Polylepidina*—this entire evolutionary transition having taken place in the lower to upper Eocene period. In this connection, it may be noted that in the present Ariyalur material, we have several remains of the Amphisteginidae, together also with a form which is probably *Helicostegina*; and the occurrence of these along with the kinds of Orbitoids described above makes the entire assemblage specially worthy of further attention.

Another interesting observation which may be briefly referred to here is that some of these Ariyalur Orbitoids on careful examination seem to show certain 'lepidocycline' features both in regard to the nucleoconch and the shape of the equatorial chambers. In his study of the Cretaceous Orbitoids of India nearly 50 years ago, Vredenburg[14] had already noticed a similar feature in the case of his *Orbitoides socialis* (from undoubted Cretaceous beds) about which he said: "One is bound to admit that the characters of this species appear undistinguishable from those of oligocene lepidocyclines; whether it represents their forerunner or only indicate a deceptive case of 'convergence' must be left to future research"; and he actually designated his form as *Orbitoides* (*Lepidocyclina*?) *socialis*.

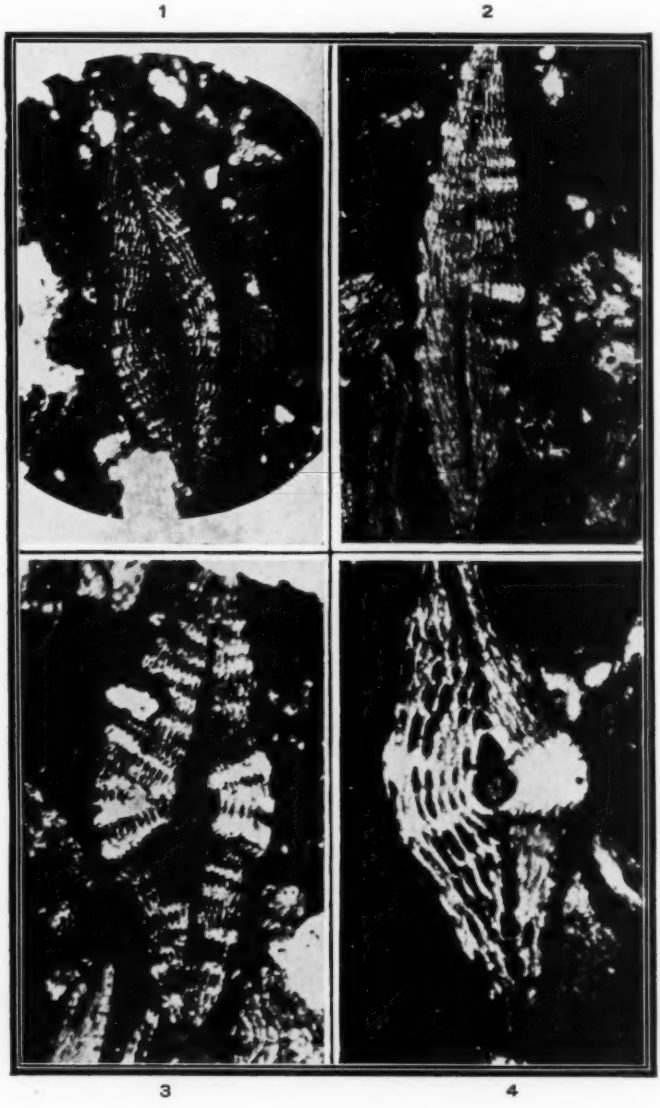
Quite recently Caudri[3] has drawn our attention to several occurrences being frequently noticed now-a-days of "small lepidocyclinoid Orbitoids in beds intermediate in age between upper Cretaceous and those in which true

lepidocyclines first appear (*viz.*, mid-Eocene), and also discussed their possible significance with special reference to the stratigraphic distribution of *Lepidorbitoides*. Some of the Orbitoids in the present Ariyalur material appear to be of the 'small lepidocyclinoid' type mentioned by Caudri, and their occurrence here in undoubted upper Cretaceous beds taken together with similar forms found in the lower part of the Tertiary elsewhere would obviously be of special interest in discussing the current ideas regarding the stratigraphic and evolutionary interrelationship between the two important genera *Lepidorbitoides* and *Lepidocyclina*.

It is hardly possible to go into the details of this very interesting problem in the course of the present paper; a few observations may however be made to indicate the importance of this line of investigation. As Caudri puts it, the genus *Lepidorbitoides* was created in 1907 by Silvestri to include "all the lepidocyclinoid forms from upper Cretaceous that were formerly included in *Orbitoides*". According to Cushman, both the genera *Lepidorbitoides* and *Lepidocyclina* belong to the same sub-family Lepidocyclinæ Tan. in the family Orbitoididae. In between these two genera we have in the Palaeocene and lower Eocene beds forms like *Orbitocyclina*, *Orbitosiphon*, *Actinosiphon* and *Polylepidina* all of which in one way or the other seem to represent transitional stages connecting *Lepidorbitoides* and *Lepidocyclina*. While most of the Orbitoids in the Ariyalur rocks answer to the description of *Lepidorbitoides*, there are some which seem to depart from the normal *Lepidorbitoides* characters and show a resemblance to one or the other Palaeocene and/or lower Eocene genera mentioned above as connecting *Lepidorbitoides* with *Lepidocyclina*. While Caudri deals with the significance of the occurrences of "Lepidocyclina-like *Lepidorbitoides*" up in the lower Tertiary, we seem to have in the Ariyalur material an example of the occurrence of small 'lepidocyclinoid' Orbitoids down in the upper Cretaceous. The detailed study of the Orbitoids included in the assemblages mentioned above will evidently be of the greatest interest and may enable us ultimately to work out the links in the evolutionary chain connecting the typical upper Cretaceous *Lepidorbitoides* at one end with the true middle Eocene *Lepidocyclina* at the other. In fact, as Storrs Cole recently says: "It is apparent to those familiar with the subject that careful revision is necessary of the various genera and subgenera at present included under the family Orbitoididae."

7. SUMMARY

The author is now engaged in a detailed study of the fossil Foraminifera from the Cretaceous rocks of South India; the present paper deals with the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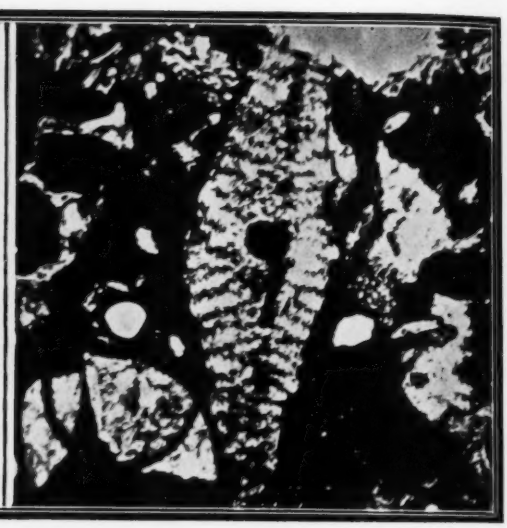

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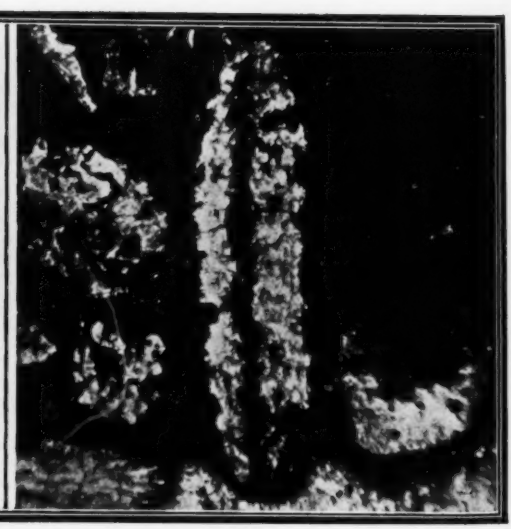

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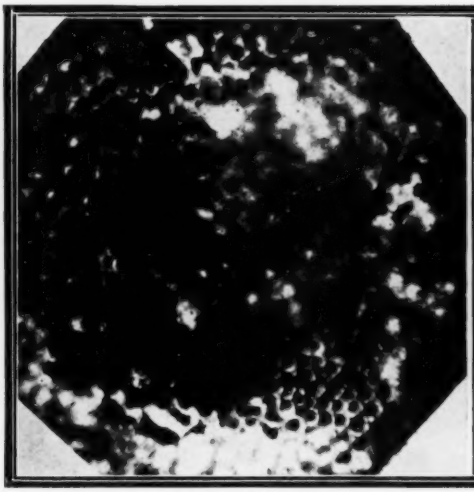

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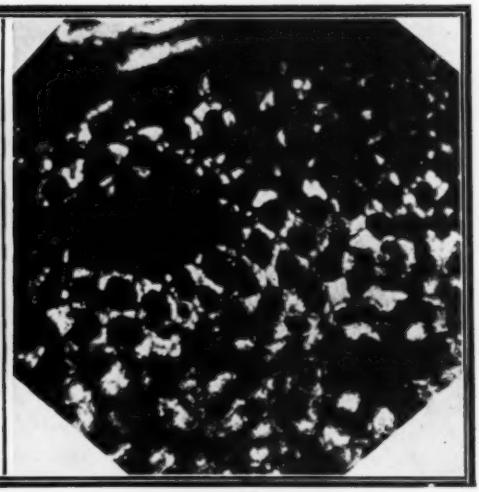

Orbitoids found in a limestone band recently noticed near Ariyalur, in the Trichinopoly area. The abundance of these Orbitoids, together with plenty of associated *Siderolites*, indicates that the containing rock is of Maestrichtian age and occupies a stratigraphical position in the Ariyalur group just below the overlying Niniyur Group (Danian). While some of the Orbitoids occurring here resemble certain species of *Lepidorbitoides* and *Orbitocyclina* already described, there are others which appear to be new. The paper gives a general account of these Orbitoids, drawing special attention to some of their interesting features. The probable significance of these in the study of the family Orbitoididae is briefly discussed.

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ADDITIONS TO THE GENUS *HANSFORDIELLA*

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Received April 5, 1957

In the course of taxonomic studies on hyphomycetes, I have come across two fungi which are now classified in *Brachysporium* but which, in my opinion, are congeneric with *Hansfordiella asterinearum* Hughes, the type species of the genus *Hansfordiella* Hughes. I am giving below descriptions of these two fungi, from type material.

1. *Brachysporium bakeri* Sydow

Brachysporium bakeri was described by Sydow (1914) as follows: " Cæspitulis hypophyllis, per totam folii superficiem sparsis v. sæpius plus minus aggregatis et intricato-confluentibus, obscure olivaceo-brunneis; hyphis conidiophoris erectis, rigidulis, remote septatis (articulis 35–55 μ long), obscure fuscis, simplicibus, 350–500 × 5–7 μ; conidiis breviter obclavatis, basi rotundatis, ad apicem obtuse attenuatis, 2-septatis, non-constrictis, dilute fuscis, levibus, guttulatis, rectis v. inæquilateria, 24–30×9–11 μ. Hab. in foliis vivis *Macarangæ* sp. (Euphorbiaceæ), Mt. Maquiling prope Los Banos, Ins. Philippin. (C. F. Baker) (Saccardo, 1931, p. 834).

I have examined type material of this fungus ex Herb. Mus. Bot. Stockholm (Herb. M.U.B.L. 1632–slide). The colonies are hypophyllous, superficial, irregular in outline, scattered or aggregated, olivaceous to brown. The repent hyphæ are subhyaline to pale brown, somewhat roughened, branched, septate and 1–3 μ wide. The conidiophores arise laterally from cells of the repent hyphæ singly or in groups of up to four, the cell from which they arise usually being swollen, of variable length, 5·6–8·4 μ wide and brown in colour. The conidiophores are erect, straight or bent, simple, cylindrical, dark brown, thick-walled, many-septate (septa being 18–54 μ apart), 4·2–8·4 μ wide at the base, 5·6–8·4 μ wide in the middle, about 7 μ wide at the tip, and 350–630 μ long. Usually, the apical part of the conidiophore up to a length of 100 μ may be sporiferous. The presence of many characteristic prominent elevated circular scars all round the sporiferous part is the chief feature of the fungus. The scars are 2·1–2·8 μ in diameter and indicate points of attachment of fallen conidia. Apart from the apical part of the conidiophore, conidia may sometimes be produced farther below

on the conidiophore and thus there may be one or more sporiferous regions on the conidiophore, other than the apical one. Sometimes, the conidiophore may proliferate at its tip through the scar of a fallen conidium, grow for a length and then produce conidia. This is one way in which more than one sporiferous region may be formed on one conidiophore. The conidia are produced singly and are acropleurogenous; they are short-obclavate, subhyaline to pale brown, 2-septate when mature, sometimes slightly constricted at the septa, broadest immediately above the base or else in the middle, tapering above, with a smoothly rounded apex, smooth-walled, 21·0–28·0 μ long, 7·7–9·8 μ wide where widest, 2·8–4·2 μ wide towards the tip, and each with a distinct basal scar 2·1–2·8 μ wide.

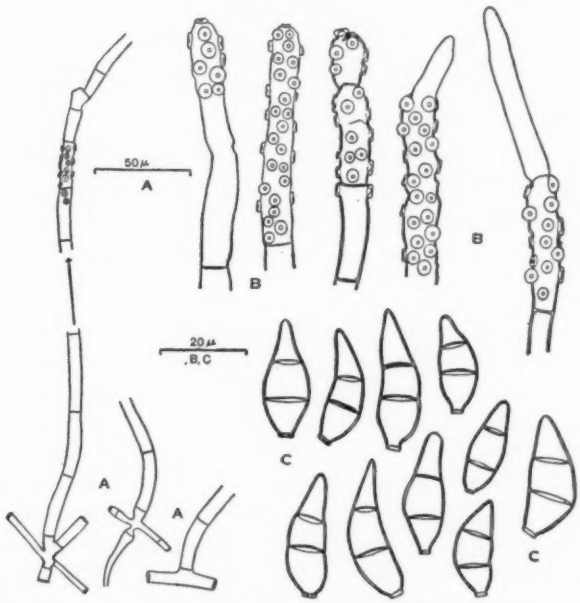

FIG. 1. *Hansfordiella bakeri* from type specimen (Herb. M.U.B.L. 1632): A, Conidiophores and their mode of origin; B, Apical sporiferous parts of conidiophore; C, Conidia.

The fungus is clearly not a *Brachysporium*; it is not congeneric with *B. obovatum* (Berk.) Sacc., the lectotype species of the generic name *Brachysporium* (see Hughes, 1951 *a*), in which the conidia develop singly at the apices of successively produced growing points and are attached to the conidiophore by a narrow, usually bent or twisted separating cell. On the other hand, the presence of many distinct and prominent circular, elevated conidial scars on the conidiophores, and the production of phæo-

phragmospores singly and acropleurogenously on the conidiophores indicate its close similarity to *Hansfordiella asterinearum* Hughes, the type species of the genus *Hansfordiella* Hughes (Hughes, 1951 *b*). Hughes characterised his genus as " in plagulis Asterinearum et Meliolæ hyperparasitantes " (Hughes 1951 *b*, p. 10), but I do not consider that *Brachysporium bakeri* should be excluded from *Hansfordiella* merely because it is not a hyperparasite on another fungus. Indeed, it has a very close resemblance to *Hansfordiella cupulifera*, as figured by Hughes (1951 *b*, p. 13). I, therefore, consider *Brachysporium bakeri* to be a good *Hansfordiella*; it is, however, specifically distinct from the three species of *Hansfordiella* so far known [*viz.*, *H. asterinearum* Hughes, *H. cupulifera* (Hansf.) Hughes and *H. meliolæ* (Hansf.) Hughes] and may, therefore, be placed in *Hansfordiella* as a separate species:

Hansfordiella bakeri (Sydow) Subramanian comb. nov.

Basonym: *Brachysporium bakeri* Sydow, 1914, *Ann. mycol.*, **12**: 201; Saccardo, 1931, *Sylloge Fungorum*, **25**: 834.

Type specimen: on *Macaranga* sp., Mount Makiling, near Los Banos, Philippine Islands, Dec. 18, 1913, C. F. Baker, 2798, ex Herb. Mus. Botan. Stockholm (Herb. M.U.B.L. 1632–slide).

2. *Brachysporium harunganæ* Hansford

Brachysporium harunganæ was described by Hansford (1946, pp. 211–12) as follows: " *Plagulæ* tenuissimæ griseæ hypophyllæ effusæ. *Mycelium* superficiale, ex hyphis subhyalinis 2–3 μ cr., indistincte remote septatis ramosis compositum, inter squamas folii repens. *Conidiophora* erecta, dispersa, simplicia, septata, atrobrunnea, subrecta, 300–500 $\times$ 6–7 μ, sursum cicatricibus atris prominulis rotundis 3–4 μ diam. laxe prædita. *Conidia* singula, acro-pleurogena, obclavata, olivacea, apice breviter attenuato-rotundata pallidiora, basi truncata, hilo plano atro, levia, 2-septata, haud constricta 30–45 $\times$ 8–11 μ. Hab. in foliis *Harunganæ madagascariensis*, Kawanda, Uganda, Hansford 3347." After the diagnosis, Hansford added: " Though the mycelial hyphæ ramify closely among the cuticular scales, no penetration of the leaf was observed, and it appears to be saprophytic in habit."

I have examined type material of this fungus ex National Fungus Collections, U.S.D.A. (Herb. M.U.B.L. 1696–slide). The colonies are hypophyllous, thin and effuse. The repent hyphæ are 2–3 μ wide, septate, branched and subhyaline to pale brown. The conidiophores arise laterally from cells of the repent hyphæ or more usually from a cell of a germinated phragmospore whose wall becomes thickened and darkened. The conidiophores are erect,

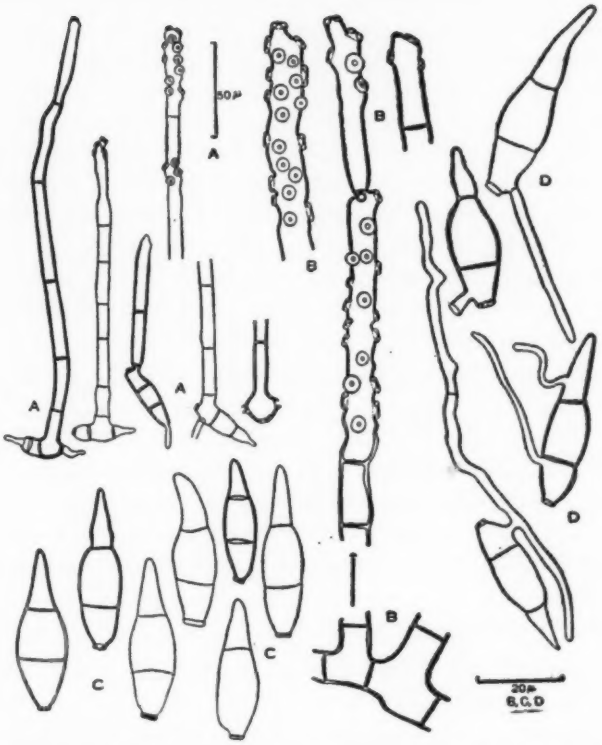

FIG. 2. *Hansfordiella harunganæ* from type specimen (Herb. M.U.B.L. 1696): A, Conidiophores and their mode of origin; B, magnified views of conidiophores and sporiferous parts; C, conidia; D, germination of conidia.

straight or bent, simple, cylindrical, sometimes with swellings, many-septate (septa 14·4–43·2 μ apart), dark brown, paler towards the tip, 322–462 μ long, 7·0–9·1 μ wide at the base, 5·6–7·0 μ wide in the middle and 6·3–9·8 μ wide towards the tip. Usually the apical part of the conidiophore up to a length of (44·8–) 126·0 μ may be sporiferous. Like *Brachysporium bakeri*, the presence of many characteristic prominent elevated circular scars on the sporiferous part of the conidiophore is the striking feature of the fungus. The scars are 3·5–5·6 μ in diameter and about 1–2 μ tall and indicate points of attachment of fallen conidia. Apart from the apical fertile part of the conidiophore, conidia may sometimes be borne farther below on the conidiophore and thus a conidiophore may have more than one sporiferous region other than the apical one. It is not unusual for a conidiophore to proliferate at its tip through the scar of a fallen conidium, grow for a length and

then produce conidia and this is one way in which more than one sporiferous region may be formed on a conidiophore. The conidia are produced singly and are acropleurogenous. They are obclavate, subhyaline to pale brown, smooth-walled, 2-septate when mature, sometimes constricted at one or both septa, usually widest in the middle, tapering above into a narrow-conical apical cell with blunt or rounded apex and 2·8–3·5 μ wide where it is widest, with the basal cell somewhat crucible-shaped and with a distinct flat, dark, basal scar 3·5–5·6 μ wide; they are 26·6–46·2 μ long and 7·7–9·8 μ wide where they are widest. They usually germinate by producing one or more germ tubes laterally from any of the three cells and at least one of them elongates, becomes thick-walled and septate and is transformed into a simple, erect conidiophore producing conidia.

It may be seen that the fungus has little similarity to *Brachysporium obovatum* since the conidia in the former are not attached to the conidiophore by separating cells; it cannot, therefore, be retained in *Brachysporium*. On the other hand, it is clear that in having prominent elevated circular scars on the sporiferous part of the conidiophore and in producing phæophragmospores acropleurogenously, the fungus has the features of a *Hansfordiella* and is similar to *Brachysporium bakeri*. Accordingly, I propose the combination:

Hansfordiella harunganæ (Hansf.) Subramanian comb. nov.

Basonym: *Brachysporium harunganæ* Hansford, 1946, *Proc. Linn. Soc. Lond.*, **157**: 211–12.

Type specimen: on *Harungana madagascariensis*, Kawanda, 3, 1944. coll. and identified by C. G. Hansford, ex Herb. Mycologist, Dept. Agric. Uganda no. 3347 ex National Fungus Collections, U.S.D.A. (Herb. M.U.B.L. 1696–slide).

3. *Helminthosporium*? *Puttemansii* Arnaud, 1953, in *Bull. Soc. mycol. Fr.*, **69**: 288.

The conidiophore, as figured by Arnaud (1953, Fig. 9 J), has prominent circular elevated scars similar to those of *Hansfordiella* spp., and Arnaud's fungus is stated to be a hyperparasite on Microthyriaceæ. I have not seen a specimen. In any case, the name *Helminthosporium Puttemansii* is a *nomen nudum* and has yet to be validated by a diagnosis.

In conclusion, I am giving below a key to the five species of *Hansfordiella* so far known and, for this purpose, details about *H. asterinearum* Hughes, *H. cupulifera* (Hansf.) Hughes and *H. meliolæ* (Hansf.) Hughes have been gathered from Hughes (1951 *b*). Of these, the first two produce phragmospores; but *H. meliolæ* produces dictyospores, but is nevertheless

classified by Hughes in this genus because of its similarity to *H. asterinearum* in other features, especially the manner of production of conidia.

KEY TO SPECIES OF *Hansfordiella*

Conidia phragmospores
  Conidiophore terminating in a single scar; conidia acrogenous .. .. .. *H. asterinearum*
  Conidiophore with many scars; conidia acropleurogenous
    Conidia 3 (-4)-septate, 34–50×7·5–9 μ .. *H. cupulifera*
    Conidia 2-septate
      Conidia 21–28×7·7–9·8 μ .. .. *H. bakeri*
      Conidia 26·6–46·2×7·7–9·8 μ .. .. *H. harunganæ*
Conidia dictyospores .. .. .. *H. meliolæ*

SUMMARY

On the basis of a study of type material, *Brachysporium bakeri* Sydow and *B. harunganæ* Hansford are excluded from the genus *Brachysporium* Sacc., as typified by its lectotype species, *B. obovatum* (Berk.) Sacc. *B. bakeri* and *B. harunganæ* are considered to be congeneric with *Hansfordiella asterinearum* Hughes, the type species of the genus *Hansfordiella* and are transferred to that genus as *H. bakeri* (Sydow) Subram. and *H. harunganæ* (Hansf.) Subram., respectively. It is suggested that *Helminthosporium*? *Puttemansii* Arnaud (*nomen nudum*) may be a *Hansfordiella*. A key to the five species of *Hansfordiella* is appended.

ACKNOWLEDGEMENTS

I am deeply indebted to Dr. J. A. Stevenson of the National Fungus Collections, U.S.D.A., Beltsville, Maryland, and to the Naturhistoriska Riksmuseet, Stockholm, for loan of specimens.

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A STUDY ON THE COMPARATIVE EFFECT OF CHEMICALS ON CHROMOSOMES OF ROOTS, POLLEN MOTHER CELLS AND POLLEN GRAINS

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Introduction

Extensive investigations have been carried out on chromosome breakages induced by chemicals (Levan and Tjio, 1948; Carr, 1950, *etc.*). Levan who has contributed much to our knowledge in this direction considers their action to be sub-narcotic in character. Apart from their practical utility, such studies are likely to throw some light on fundamental problems also. The reaction of different chromosome segments to a particular chemical gives an indication of the differences in the same chromosome. Localised breakages occurring in the region of the secondary constrictions in *Vicia faba* are considered to signify that they differ from the other chromosomal regions.

In spite of the large amount of work on induction of chromosome breakages by chemicals, little attention has been paid to the response of the chromosomes from the germinal and somatic tissues of a plant to the same chemical. It is known that a good fixative for the root tips, *e.g.*, Lewitsky's fluid, is often unsuitable for a study of the meiotic stages. Mitotically dividing cells from different tissues show often differences in response to the same fixative. Such records would imply that there is considerable variation in the chemical make-up of the chromosomes themselves.

Mitotic chromosomes from soma have been the favourite material for an evaluation of the effects induced by various chemicals. Pollen grains have not interested investigators in this line, nor has there been any attempt at comparison of the effect of the same chemical on somatic and meioitc cells. Their differential responses, if any, would give an indication of the qualitative and quantitative differences in the make-up of the chromosomes as also the suitability or otherwise of particular types of cells for such investigations.

The present investigation was undertaken to evaluate the effect of three different chemicals on mitotic and meiotic cells of *Nothoscordum fragrans* Kunth.

Materials and Methods

1. *Materials*

The species utilized in the present investigation is *Nothoscordum fragrans* Kunth. It was grown in sawdust and garden soil mixture (1 : 1) in flower pots and also in soil at the University College Compound at Ballygunge, Calcutta.

Root-tips, flower-buds and mature pollen grains of this species were utilized for the investigation.

2. *Chemicals studied for their comparative effects on different mitotically and meiotically dividing cells*

(*a*) Aesculine,

(*b*) Paradichlorobenzene, and

(*c*) Resorcinol.

Solutions were prepared in distilled water. Aesculine and Paradichlorobenzene were used as saturated solutions. Resorcinol was applied in 0·1 mol. solution.

3. *Method of application of chemicals*

(*a*) *In root-tips.*—The root-tips were treated separately in different chemical solutions at a temperature of 10–14° C. and the period of treatment was varied in each experiment.

(*b*) *For the study of Meiosis in P.M.C.'s in flower-buds and in mature flowers for pollen mitosis.*—(i) *By injection*: The solution (1 c.c.) was taken in a glass syringe and injected into the inflorescence axis. It was noted that more than 1 c.c. of the solution could not be injected.

(ii) *By dipping the inflorescence tip in the solution.*—The tip of the inflorescence while still attached to the plant was kept immersed for differing periods in a solution of the chemical in a petri dish either at the room temperature (26° C.) or at slightly lower temperatures ranging from 10–14° C.

In addition to this, experiments were also carried out in which inflorescence tips with buds and mature flowers were treated after removal at a temperature of 10–14° C. for varying periods in the solution.

4. *Method of staining*

(*a*) *Root-tips.*—After treatment, the root-tips were fixed and stained in Aceto orcein (2%): Hydrochloric acid (N) mixture (9: 1) by slight heating and smeared in Aceto orcein solution (1%) to study the effect of different chemicals used.

(*b*) *Flower-buds and mature flowers.*—After treatment, the anthers of suitable size were taken and smeared in Aceto carmine (1%) to study the effect of the chemical on meiotic chromosomes.

In order to evaluate the effect of the chemical on chromosomes of pollen grains, anthers of suitable size were smeared and fixed in Nawaschin's fluid as usual. Staining was carried out with the Crystal Violet technique.

Temporary smear preparations of dividing pollen grains with Aceto-carmine (1%) were also used.

All the diagrams were drawn with *a* × 12 eyepiece and an 1·3 apo-chromatic objective with a condenser 1·4 N.A. at a table magnification of approximately, × 2,000.

OBSERVATIONS

Effect on mitotically dividing cells

(*a*) *On root-tips.*—In the smear preparations, divisional figures and specially the metaphase and anaphase stages were carefully examined to study the effect of the chemical on the chromosomes. Large number of dividing cells were observed, thereby indicating no antimitotic action at the initial stage. Considerable erosion and fragmentation of chromosomes occurring at random were noted both in metaphase and in anaphase. In comparatively lower concentrations, the number of fragments was found to be two to three and in higher concentrations the number was six to ten. Cells with a higher number of fragments could obviously divide no further. It was observed that comparatively longer chromosomes were fragmented more than the smaller ones (Plate XXX, Fig. 1).

(*b*) *On pollen grains.*—After treatment, pollen grain chromosomes were studied. Diplochromosome formation was detected, but in no case was fragmentation of chromosomes observed even after prolonged treatment (Plate XXX, Fig. 2).

Effect on meiotic cells

After chemical treatment of meiotic cells, irregularities in division, such as lagging, non-disjunction and stickiness were detected, but in no case

was fragmentation of chromosomes observed even after prolonged treatment (Text-Figs. 1 and 2).

TEXT-FIGS. 1 and 2. Meiotic plates of *N. fragrans* Kunth. showing non-disjunction and agging of chromosomes after prolonged (3 hours) treatment in Resorcinol (0·1 ml.).

The results obtained are summarised in Tables I and II.

TABLE I

Effect noted after short period of treatment

Chemicals used	Time of treatment	Root-tips		Flower-buds	
		No. of fragments in		Meiotic cells	Pollen grains
		Metaphase	Anaphase		
Aesculine	1 hr.	2–3	1–3	Only stickiness of chromosomes noted	No irregularity in chromosomes noted
Paradichlorobenzene	3½ hrs.	1–2	1–2	Do.	Do.
Resorcinol	1 hr.	2–3	2–3	Do.	Do.

From the tables it is apparent that root-tip chromosomes undergo fragmentation, whereas pollen grain chromosomes do not show any fragments. Meiotic cells too do not reveal the presence of fragments. By increasing the period of treatment, the number of fragments goes on increasing in root-tip nuclei, but still pollen grain chromosomes do not undergo any fragmentation.

DISCUSSION

It has been emphasised in the text that not all the mitotic cells behave in a similar way. Their different behaviour is apparent even after fixation

TABLE II

Effect noted after prolonged treatment

Chemicals used	Time of treatment	Root-tips		Flower-buds	
		No. of fragments in		Meiotic cells	Pollen grains
		Metaphase	Anaphase		
Aesculine	3 hrs.	6–10	6–8	Lagging, non-disjunction and stickiness of chromosomes but no fragments noted	No fragmentation, but diplochromosomes in few cases
Paradichlorobenzene	6 hrs.	5–8	5–8	Do.	Do.
Resorcinol	3 hrs.	7–10	6–10	Do.	Do.

in the same chemical. The present investigation brings out clearly the differential response of chromosomes of pollen grains and root-tips to specific chemicals.

With all the chemicals, root-tip chromosomes undergo heavy fragmentation, whereas the mitotically dividing chromosomes of pollen grains remain almost unaffected. It is a positive indication of the differential set-up of the chromosomes of these two types of cells. At the same time erosion and stickiness in root tips are not uncommon. Evidently all these are brought about by a disturbance of the nucleic acid metabolism of the cell. The number of fragments goes on increasing with increase in the period of treatment.

Pollen grain chromosomes rarely show any response. Only in certain cases were diplochromosomes noted. This indicates a slight disturbance in the spindle mechanism and a failure of chromosome separation.

It seems likely that the metabolism of the chromosomes of pollen grains and root-tips, may to some extent be different. The protein and nucleic acid synthesis of the chromosomes may be somewhat different in the two. Such a suggestion does not naturally involve the assumption of fundamental chemical differences in chromosome structure.

FIG. 1. Photomicrograph of somatic metaphase plate of *Nothoscordum fragrans* Kunth. when treated in Resorcinol (0·1 mol.) for 1 hour showing fragmentation of chromosomes.

FIG. 2. Photomicrograph of pollen grain metaphase of *N. fragrans* Kunth. showing 10 chromosomes and no fragment after prolonged (3 hours) treatment in Resorcinol (0·1 mol.).

As regards meiotic chromosomes, irregularities not involving fragmentation have been recorded. Such irregularities may arise out of a disbalance of the nucleic acid cycle and a disturbance of the spindle mechanism. The absence of fragmentation in meiotic cells may possibly be due to differences in their metabolic set-up as compared to the mitotic ones as also the physical state of the chromosomes and the bivalents themselves.

Summary

1. A comparative study of the action of Aesculine, Paradichlorobenzene and Resorcinol on the root-tip, pollen grain and pollen mother cell chromosomes of *Nothoscordum fragrans* Kunth. reveals considerable erosion and fragmentation at meta- and anaphase in root-tips.

2. The division is mitotic in pollen grains but yet prolonged treatment resulted only in diplochromosome formation and not chromosome fragmentation. Fragmentation was also not observed in meiotic cells. Chemical treatment produced only irregularities in division such as lagging, non-disjunction and stickiness.

3. The marked differences in response to chemicals between the root-tip cells on the one hand and the pollen grains and P.M.C.'s on the other is suggestive only of differences in chromosome metabolism and not the ultimate structure of the chromosomes.

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CHROMOSOME NUMBERS IN INDIAN MEDICINAL PLANTS

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The Flora of India more than that of any other country in the world, abounds in plants which are of medicinal value to both man and beast. These plants have been collected and utilized from very ancient times and our present knowledge is based on the intelligent observation and inquiry of our ancestors.

Minute morphological differences in plants like the presence or absence of hair and the thickness of leaves or the size of flowers, have been the means of differentiating species. It is only on the correct identification of the plant that the authencity and utility of the drug depends, and today with the advance of Botany it is possible to identify plants with the use of a modern Herbarium. However, it has often been shown that the medicinal properties of a plant are dependent on the habitat of the plant. Studies of medicinal plants from different habitats becomes important especially when we propose to grow drug plants on a large scale as in the case of *Rauvolfia* or *Emblica*.

The active principles of a plant vary not only with the different seasons and with the different environments the plant is grown but they also depend on the "ploidy" of the plant. It often occurs that the same species of a genus may have different polyploid varieties. Such polyploids owing to the duplication of genes may also be richer in medicinal and other properties. The content of vitamin C in triploid apples and tetraploid tomatoes is markedly higher than in the diploid. Thus, there is a direct correlation between polyploidy and vitamin content. In the same way the ephedrine content of the various polyploid species of *Ephedra* is on the whole higher than that of the diploid (Janaki Ammal, unpublished). The study of genetic variation in medicinal plants becomes important before selecting the right variety to cultivate, and a knowledge of the chromosome numbers of medicinal plants is of primary importance.

In this paper the chromosome numbers of some of the genera and species of medicinal plants which are being cultivated in the Central Botanical

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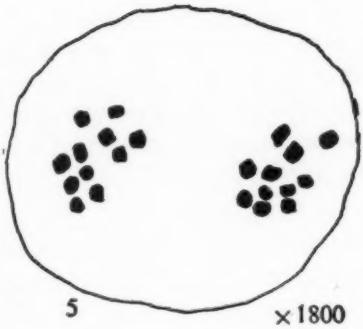

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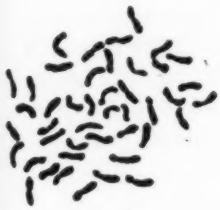

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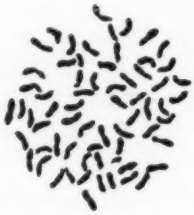

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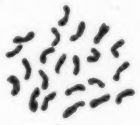

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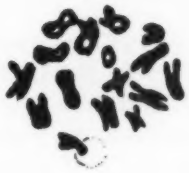

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Laboratory are given and variation in chromosome numbers have been noted wherever they have been observed by others.

The observations of the plants have been made mainly from root tips. These were fixed in chrome-acetic-formalin, sectioned at 12 μ and stained in crystal violet. Where the plants flowered, pollen mother cells have also been studied. The buds were fixed in acetic alcohol and squashed in aceto-carmine. Camera lucida drawings were made and the appropriate magnification is given at the end of the drawings.

Of the twenty plants examined, the chromosome numbers of nine plants have been reported for the first time and these have been marked with *. Five plants marked ** show numbers different from previous observations. In the remaining six plants the chromosome numbers are the same as reported by previous workers (*vide Chromosome Atlas of Flowering Plants*, by Darlington and Wylie, 1955).

The present study has revealed the presence of polyploidy for the first time in *Emblica officinalis* and in the genus *Rauvolfia*. In *Emblica officinalis* a high polyploid ($7x$) has been discovered in a Lucknow variety. Perry (1943) has reported the number of the diploid as $2n = 28$. In the three species of *Rauvolfia* studied there is an interesting grade of polyploidy indicating the basic number of the genus as $x = 11$. Both *Calotropis procera* and *C. gigantea* have $2n = 22$ indicating the basic number of the genus as $x = 11$. It is interesting to note that *Hemidesmus indicus* also has a basic number of $x = 11$. Thus $x = 11$ is the most common basic number for the family *Asclepiadaceæ*. In *Aegle marmelos* the diploid ($2n = 18$) form which was not known before has now been found in the wild from the Vindhyas as well as in plants grown in Lucknow.

Ghimpu (1929) has reported the diploid number of *Cissus quadrangularis* as *ca* 45 but present observations have shown $2n = 24$ chromosomes in the Indian form. It is probable that the plant examined by Ghimpu might be a tetraploid one. The diploid number of $2n = 18$ reported for *Curculigo orchioides* ($2n = 18$) in the present paper agrees with the findings of Sheriff (1946). Sato (1938) has reported $2n = ca$ 50 for the same species. Polyploidy may account for the difference in number.

In *Cuscuta reflexa* Finn (1937) has reported $2n = 28$ ($x = 7$) but observations in the Lucknow plant has revealed $n = 16$ only. Thus a new basic number ($x = 8$) is found occurring in the same species. It is interesting to note that the North American *Cuscutas* are reported to have a basic number of $x = 15\,(7 + 8)$.

The following is the list of chromosome numbers of medicinal plants studied :—

Family and Name	Vernacular Name	Present Observation
MORINGACEÆ		
Moringa pterygosperma Lam.	Sainga	$2n = 28$
POLYGONACEÆ		
Rumex dentatus L.	..	$n = 20$
EUPHORBIACEÆ		
**Phyllanthus niruri* L.	Jangli amali	$2n = 26$
***Emblica officinalis* Gaertn.	*Amla, amlika*	$2n = 98$
CAESALPINACEÆ		
Bauhinia purpurea L.	Koiral, Karalli	$2n = 28$
AMPELIDACEÆ		
***Cissus quadrangularis* L.	Hasjora	$2n = 24$
RUTACEÆ		
***Aegle marmelos* Corr.	Bæl	$2n = 18$
**Toddalia asiatica* Lamk.	Dahan, Kanj	$2n = 72$
Murraya kænigii Spreng.	Katnim	$2n = 18$
Murraya exotica L.	Marchula	$2n = 18$
APOCYNACEÆ		
**Rauvolfia serpentina* Benth.	Sarpagandha	$n = 11$ $2n = 22$
**R. densiflora* Benth.		$2n = 44$
**R. canescens* L.		$2n = 66$
ASCLEPIADACEÆ		
**Calotropis procera* R. Br.	Ak, Madar	$2n = 22$
**C. gigantea* R. Br.	Ak, Akra	$2n = 22$
**Hemidesmus indicus* R. Br.	Anantmul	$2n = 22$
COMPOSITÆ		
Xanthium strumarium L.	Chhotagokhru	$n = 18$
CONVOLVULACEÆE		
***Cuscuta reflexa* Roxb.	Akasbela	$n = 16$
ACANTHACEÆ		
**Andrographis paniculata* Nees.	Kalomegh, Kiriat	$n = 25$
HYPOXIDACEÆ		
***Curculigo orchioides* Gaertn	Kalimusli, Muslikand	$2n = 18$

SUMMARY

The paper deals with the chromosome numbers in some of the Indian medicinal plants. Of the twenty plants studied, the chromosome numbers of nine plants are reported for the first time. Five plants had numbers different from previous observations.

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EXPLANATION OF TEXT-FIGURES

TEXT-FIGS. 1–12. Fig. 1. Somatic metaphase of *Phyllanthus niruri* showing $2n = 26$ chromosomes. Magnification, × 2,800. Fig. 2. Somatic metaphase of *Cissus quadrangularis* showing $2n = 24$ chromosomes. Magnification, × 2,800. Fig. 3. Somatic metaphase of *Aegle marmelos* showing $2n = 18$ chromosomes. Magnification, × 2,800. Fig. 4. Somatic metaphase of *Toddalia asiatica* showing $2n = 72$ chromosomes. Magnification, × 2,800. Fig. 5. Metaphase II of *Rauvolfia serpentina* showing 11 chromosomes. Magnification, × 1,800. Fig. 6. Somatic metaphase of *Rauvolfia serpentina* showing $2n = 22$ chromosomes. Magnification, × 2,800. Fig. 7. Somatic metaphase of *Rauvolfia densiflora* showing $2n = 44$ chromosomes. Magnification, × 2,800. Fig. 8. Somatic metaphase of *Rauvolfia canescens* showing $2n = 66$ chromosomes. Magnification, × 2,800. Fig. 9. Somatic metaphase of *Calotropis procera* showing $2n = 22$ chromosomes. Magnification, × 2,800. Fig. 10. Somatic metaphase of *Calotropis gigantea* showing $2n = 22$ chromosomes. Magnification, × 2,800. Fig. 11. Somatic metaphase of *Hemidesmus indicus* showing $2n = 22$ chromosomes. Magnification, × 2,800. Fig. 12. Diakinesis of *Cuscuta reflexa* showing 16 bivalents. Magnification, × 700.

ROOT EXUDATES IN RELATION TO THE RHIZOSPHERE-EFFECT

By (Miss) K. Bhuvaneswari and N. S. Subba-Rao

(*University Botany Laboratory, Madras-5*)

Received May 3, 1957

(Communicated by Prof. T. S. Sadasivan, F.A.SC.)

Introduction

In the course of analyses of rhizosphere microflora of several crop plants, it was observed that while sorghum plants (*Sorghum vulgare*, var. *dochna*) showed increased microbial population of the rhizosphere (positive rhizosphere-effect), mustard plants (*Brassica juncea*) showed a definite decrease (negative rhizosphere-effect) when compared to the microbial numbers of the unplanted soil. Since it is now generally accepted that stimulation or inhibition of micro-organisms around roots depend on the nature of root exudates, a study of root excretions of these plants was made and reported hereunder.

Materials and Methods

Root exudates from 4-day old seedlings were collected by the technique described earlier from this laboratory (Andal, Bhuvaneswari and Subba-Rao, 1956) and examined for the presence of organic acids and sugars by the circular paper chromatographic technique (Lakshminarayanan, 1954), using *n*-Butanol/Acetic acid/Water::4:1:5 for sugars and *n*-Butanol/Formic acid/Water::10:2:5 for organic acids as the developing solvent mixtures. The developed chromatograms were sprayed with B.D.H. 4·5 indicator for detection of organic acids and aniline hydrogen phthalate for sugars (Patridge, 1949).

Results and Discussion

Several organic acids and sugars were present in the root exudates of both the plants (Table I). Of these tartaric and oxalic acids were common to both sorghum and mustard; likewise, *d*-xylose and *d*-fructose were present in the exudates of both the plants. However, mustard had malic and citric acids, *d*-glucose and maltose in addition.

It is now known that plant roots exude various amino acids (Katznelson, Rouatt and Payne, 1955; Parkinson, 1955; Rovira, 1956; Andal *et al.*, *loc. cit.*), vitamins (West, 1939; Bhuvaneswari and Sulochana, 1955), flavones

Table I

Organic acids and sugars in the root exudates of sorghum and mustard

Exuded organic acids and sugars	Mustard	Sorghum
Malic acid	+	−
Citric acid	+	−
Tartaric acid ..	+	+
Oxalic acid	+	+
d-xylose	+	+
d-fructose	+	+
d-glucose	+	−
Maltose	+	−

+ = Present. − = Absent.

and nucleotides (Lundegårdh and Stenlid, 1944) and hydrocyanic acid (Timonin, 1941). The present report, however, has demonstrated the liberation of organic acids and sugars by actively growing roots. It is quite possible that malic and citric acids contained in the root exudate of mustard might be responsible for the observed negative rhizosphere-effect of these plants and indeed intensive work on the role of these organic root excretions in controlling the microbial population of the rhizosphere is bound to be fruitful.

Summary

Chromatographic study of the root exudates of *Sorghum vulgare*, var. *dochna* (Sorghum) and *Brassica juncea* (Mustard) revealed the presence of several organic acids and sugars in them. Although tartaric and oxalic acids, *d*-xylose and *d*-fructose were common to both, mustard had malic and citric acids, *d*-glucose and maltose in addition. The possibility of malic and citric acids as factors influencing the observed depression of the rhizosphere microflora of mustard plants has been suggested.

Acknowledgments

We are grateful to Prof. T. S. Sadasivan for guidance and Dr. C. V. Subramanian for his keen interest in the present investigation.

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THE ROUTINE-ACTIVE OXYGEN CONSUMPTION OF THE MILK FISH

By S. V. Job

(*Central Marine Fisheries Research Station, Mandapam Camp*)

Received April 20, 1957

(Communicated by Dr. N. K. Panikkar, F.A.Sc.)

Introduction

Seasonal variations in temperature, dissolved oxygen and salinity have great influence upon marine life. However, these factors incidental to the tropical environment have not been fully investigated. In this paper some effects of a sudden change in temperature as well as the effects of various partial pressures of oxygen upon its uptake are presented with reference to *Chanos chanos*—the milk fish, so widely used in fish culture. An attempt has also been made to relate this study to a limited range of size of the fish.

A measure of the metabolism or energy turnover of the fish is necessary in order to estimate the requirements for promoting and sustaining growth. Since any change or regulation of metabolism is directly reflected in respiration, the uptake of oxygen by the fish is taken as a measure of its metabolism. Two levels of metabolism are usually recognised: *Active* metabolism which is the total or maximum that the animal is capable of, and *Standard* which is the measurable minimum required for sustaining all ancillary activities of life, below which level the animal is bound to die sooner or later. As established by Fry (1947), the 'scope for activity' of the animal is conditioned by the relation the two metabolic levels bear to each other. This indeed has been demonstrated in various ways by Spoor (1946), Fry and Hart (1948), Graham (1949), and Job (1955); and the importance of relating oxygen consumption precisely to the activity of the fish in similar studies needs no further emphasis. In this investigation, because of limitations in technique the fish could be kept in a routine state of activity only and were obviously not executing any consistently active swimming movements, hence the term 'routine-active' is employed here.

Duodoroff (1942, 1945), Brett (1944, 1952) and Fry (1947) have shown that thermal adaptation both in nature and in experimental conditions is related to the previous temperature history; and that, from this experience the fish acquires various degrees of ability to cope with the temperature conditions of the environment. Brett and Fry have described this effect as

'acclimation' and the term has been used in this paper also, to convey the same idea.

Viswanathan and Tampi (1952), working on the same species measured the oxygen consumption of various sizes and at different temperatures. But the measurements were not related to any consistent size-range at the different temperatures. It was mainly to probe into some unsuspected and interesting aspects of this complex problem and to supplement their data, that this investigation was undertaken.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. N. K. Panikkar, Chief Research Officer of this Station, for his interest in this work. Thanks are also due to Miss N. G. Sproston for critical reading of the paper and to Shri D. V. Thampuran for confirming the statistical procedure adopted. The help rendered by Shri P. Balaraman and Shri M. Kasim in the collection and maintenance of the material is gratefully acknowledged.

MATERIAL AND METHODS

Fry of *Chanos chanos* were obtained from tidal creeks in Pamban and were reared in large glass tanks with an adequate supply of tap water of low salinity (0·3‰). They were fed with unlimited quantities of standard food consisting of sheep's liver, clams and seaweeds mixed in definite proportions and ground into a paste. Even though the temperature of water fluctuated over the year, the experiments were so timed that the daily temperature for over a month remained around 29 $\pm$ 1° C., before and during the experimental period. Fishes weighing from 0·7 gm. to 11·0 gm. were used in the experiments.

The respiration chambers, Fig. 1, consisted of culture bottles and Ehrlenmeyer flasks, 50 ml. to 5,000 ml. in capacity. The size of the vessel used was such that the enclosed fish was quite free to swim round. The chamber was closed with a rubber stopper carrying both the inlet (1) and the outlet (2). The former was a burette, which by opening the stopcock, would deliver through a long capillary (3) a measured volume of water to the bottom of the chamber. At the time of sampling, the outlet (2) which was normally kept plugged was opened so that an equal volume was simultaneously expelled. Samples were taken at 10, 20, 30 and 60 minute intervals. The chambers were held in water-baths at 25° C. and 29° C. and maintained constant to $\pm$ 0·2° C., throughout the duration of the experiment. Unmodified Winkler technique was employed for measuring dissolved oxygen and 5 ml. or 10 ml. samples were usually analysed. The respiration chambers were

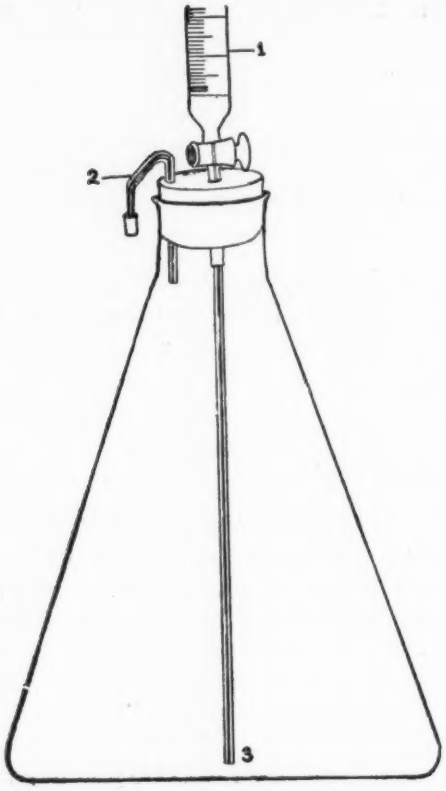

FIG. 1. A simple apparatus for measuring the routine-active oxygen consumption of a fish. 1 and 3. Water inlet. 2. Water outlet.

agitated by the running motor of an air-compressor unit and this kept the fish reasonably active. Some minutes before sampling the motor was turned off so that the fish became more quiescent and did not disturb the water in the chamber while the sample for analysis was actually being drawn. By using coloured water under identical conditions and by repeated tests, the above procedure was adopted so that the water drawn off for sampling did not become contaminated with the water that was introduced, and it represented only the true sample from the chamber.

The pH was measured both at the beginning and at the end of the experiment and the change was always less than 1·0 in the range 8·5 to 7·2. The total volume of water in the chamber was measured and the fish were weighed. The fish were used individually in each experiment. The rate of

oxygen consumption was calculated from the rate of fall in the oxygen level in the sample, with necessary corrections, and expressed in ml./hr. Owing to an unforeseen accident, the stock of fish was lost and the data obtained at 25° C. suffered in consequence and work at other temperatures had to be abandoned. The oxygen consumption data obtained in preliminary experiments showed that *Chanos* is dependent upon the oxygen tension as it obtains under air saturation (approximately 150 mm. Hg) both at 25° C. and 29° C. In order to extend this study into the region of ' non-dependence ' (Job, 1955), the oxygen tension was raised to a maximum of 300 mm. Hg. However, unlike the results of Wiebe (1933) and Leiner (1938) there was no reduction in the oxygen uptake under high tensions of the gas. This was probably the effect of keeping the fish uniformly active as has been noted in the speckled trout by Job (1955).

Results

The data were plotted on a double logarithmic grid of oxygen consumption against weight (Fig. 2). In Panel A are the data obtained at 29° C. which was both the acclimation and experimental temperature. In Panel B, are the data from fish at 25° C., which were acclimated to 29° C. The partial pressure of oxygen is given at the upper end of each curve. The regression values for the curves in Panel A, from 300 mm. Hg to 25 mm. Hg are:

$Y = 0{\cdot}3675 + 0{\cdot}7952x$; $Y = 0{\cdot}2308 + 0{\cdot}8236x$;

$Y = 0{\cdot}1970 + 0{\cdot}8242x$;

$Y = 0{\cdot}1567 + 0{\cdot}8124x$; $Y = 0{\cdot}0995 + 0{\cdot}7765x$;

$Y = 0{\cdot}0058 + 0{\cdot}6849x$; and

$Y = -0{\cdot}6181 + 0{\cdot}7746x$, respectively. The corresponding values for Panel B are:

$Y = -0{\cdot}0034 + 0{\cdot}9573x$; $Y = -0{\cdot}2521 + 1{\cdot}0615x$;

$Y = -0{\cdot}3452 + 1{\cdot}0842x$;

$Y = -0{\cdot}4895 + 1{\cdot}1310x$; $Y = -0{\cdot}7141 + 1{\cdot}2003x$;

$Y = -0{\cdot}9651 + 1{\cdot}2358x$; and

$Y = -1{\cdot}0258 + 0{\cdot}8460x$, respectively. These values were derived from the formula $Y = bx^k$, where Y is the log. rate of oxygen consumption, x the log. of weight, b the slope of the line and k, a constant.

DISCUSSION

The data in Fig. 2 when replotted and illustrated as in Figs. 3 and 4 bring out clearly the relationship of size to two different temperatures and to various partial pressures of oxygen in the medium respectively.

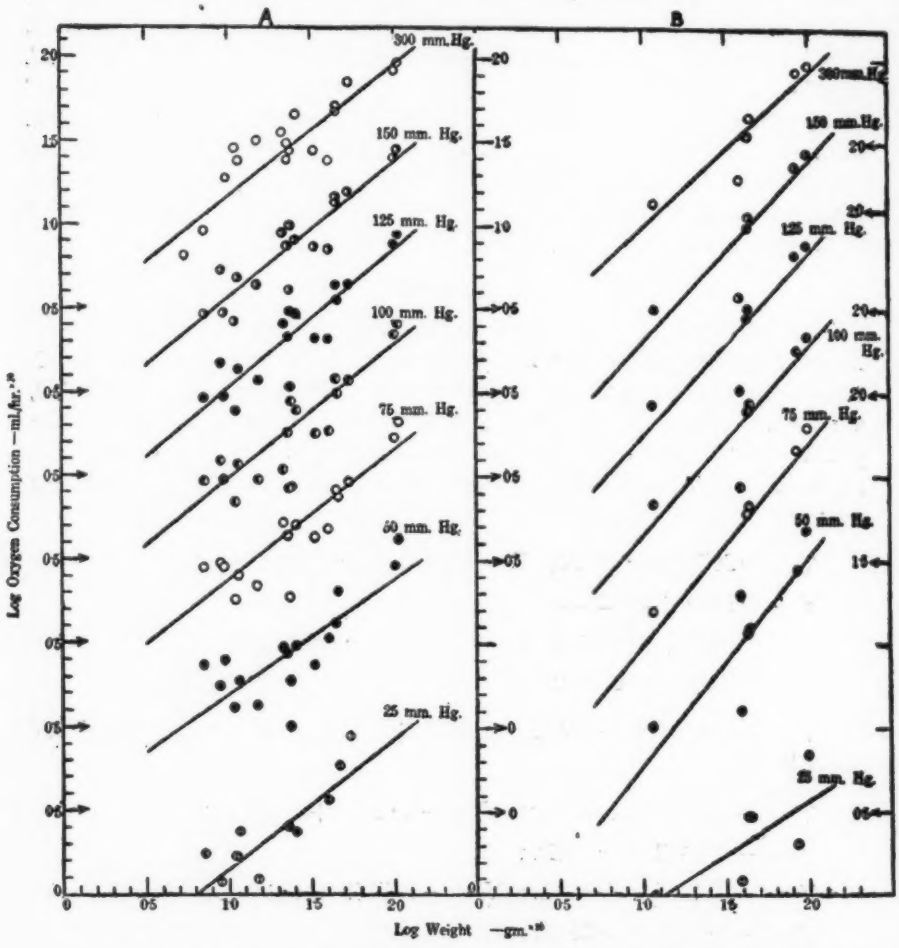

FIG. 2. Data of routine active metabolism at 29° C. (Panel A) and at 25° C. (Panel B). The partial pressures of oxygen are indicated at the upper end of each line. The lines of best fit were drawn according to the principle of least squares. The arrows along the ordinate indicate in this double logarithmic plot, the corresponding cycle of the line immediately opposite.

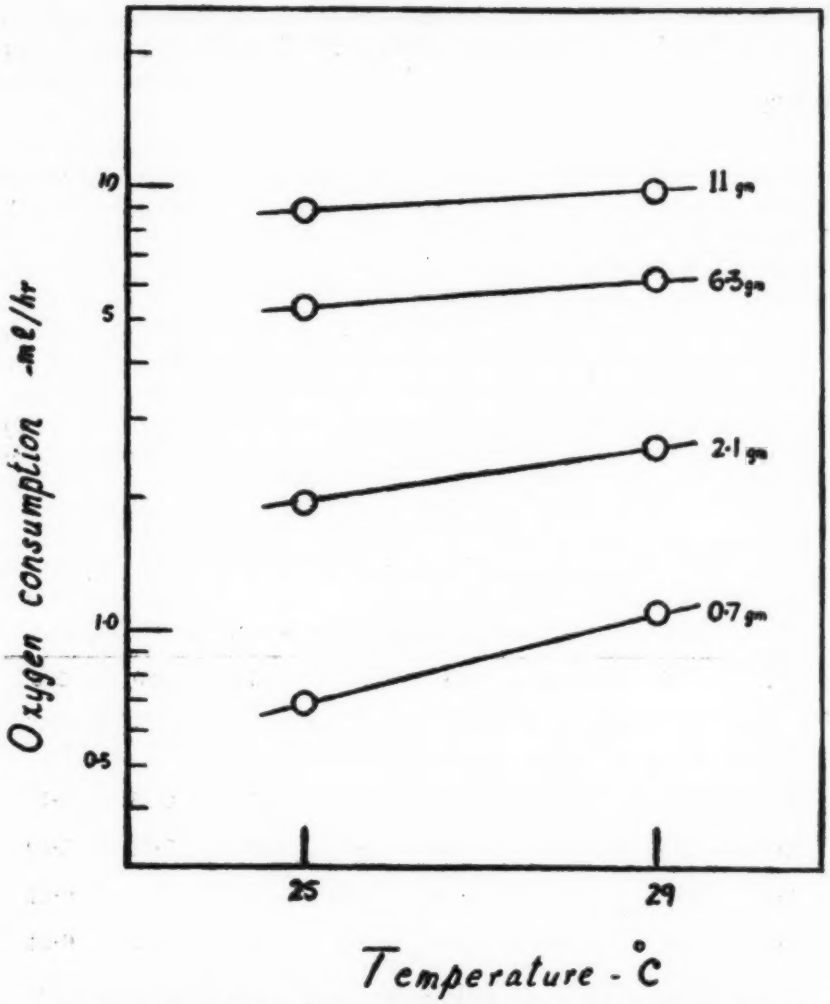

FIG. 3. Illustration of size and temperature relationship in 'routine-active metabolism' at an average pO_2 of 300 mm. Hg. At the upper end of each line is given the respective weights of the fish which were taken in logarithmic intervals except for the last group (11 gm.).

Size and Temperature

The data obtained for the milk fish bear out in a limited range of size and temperature, the trend obtained in experiments on the speckled trout (Job, 1955), wherein the value of the gradients increased as the temperature

decreased from 20° C. to 5° C. A conspicuous rise in the regression-gradients was obtained at 25° C. compared with those at 29° C. in this work: the former gradients ranging from 0·8460 to 1·2358 and the latter from 0·6849 to 0·8242.

There is, further, a remarkable 'acclimation-effect': the relative response in the metabolic rate at 29° C. (Fig. 2, Panel A) has a regularity which however is not true of the set at the unacclimated temperature of 25° C. (Panel B). Even though the difference is only 4° C., the lower temperature, because of the suddenness of impact upon fish acclimated to the higher temperature, acts probably as an accessory factor producing various effects on the different sizes (Table I). In studies of this nature therefore, the previous history of the organism in relation to any particular factor of the environment under investigation cannot be overlooked. Comparison of data obtained at various arbitrary temperatures (Viswanathan and Tampi, 1952) will not therefore give reliable pictures of the metabolic responses of the various sizes.

TABLE I

Oxygen Consumption ml/hr.

Weight gm.	Partial Pressure of Oxygen mm. Hg						
	25	50	75	100	125	150	300
			At 29° C.				
0·7	0·11	0·38	0·57	0·71	0·78	0·85	1·11
2·1	0·26	0·82	1·34	1·73	1·93	2·11	2·60
6·3	0·60	1·74	3·16	4·17	4·84	5·13	6·22
11·0	0·91	1·40	4·84	6·50	7·69	8·07	9·84
			At 25° C.				
0·7	0·07	0·12	0·20	0·30	0·37	0·44	0·69
2·1	0·14	0·46	0·74	1·00	1·23	1·41	1·95
6·3	0·27	1·82	2·82	3·55	4·07	4·57	5·37
11·0	0·39	3·55	5·37	6·61	7·41	8·13	8·91

At 25° C., while there is a slight fall in the over-all metabolic rates compared with those at 29° C. (Table I), for all sizes, the smallest fish shows the greatest change in the rate of uptake between the two temperatures (Fig. 3). Sumner and Duodoroff (1938) report that reversal of metabolic response takes place when fish acclimatized to high and low temperatures are tested at some intermediate temperature. They state further that heat-resistance did not show any size relationship. This is also largely true in the Pacific salmon as was demonstrated by Brett (1952). But he established that death from low temperature (30% to 50% mortality) was significantly higher in the smaller fish. He suggested that this was due to the greater susceptibility of smaller fish to ' rapid ' cold death. The data on the speckled trout (Job, 1955) gave a different view of this temperature-response in fish according to size. Though tentative, it was demonstrated that the ' maintenance costs ', that is the % of standard over active metabolism, was relatively higher for the smaller fish than for the bigger fish at the lower temperature. In the milk fish it seems highly probable that the greater dependence of the smallest fish, even at 300 mm. Hg pO_2 (Fig. 4), is due to the higher maintenance costs at the lower temperature and this probably also accounts for the difference in response by size noted by Brett in the Pacific salmon. In the absence of exact measurements of standard metabolic rates for the milk fish this explanation is offered with reservation.

Size and Oxygen Tension

Another point of interest can also be presented by tracing the relation of size to the ability of the fish to utilise oxygen and the oxygen tension in the medium. As can be seen clearly in Fig. 4, Panel A, when the acclimation temperature and the experimental temperature are identical, the relative ability of the fish to utilise oxygen is the same irrespective of size. The curve for the 11 gm. fish when reduced and superimposed upon that of the 0·7 gm. fish coincides with it along almost the entire range, thereby indicating the similarity in the capacity for oxygen utilisation under all partial pressures. This was also noted in the speckled trout (Job, 1955), and over a wider range in size and temperature. When an accessory factor is introduced, such as in this case a sudden fall in temperature, this relationship does not hold good, as can be seen from Panel B, in the same figure. In the former case (Panel A) the difference in the % of fall in the rate of oxygen uptake at the highest and lowest partial pressures of the gas, between the 11 gm. and 0·7 gm. fish, was 0·66%. In the latter case (Panel B) the corresponding fall was greater, amounting to 5·77%.

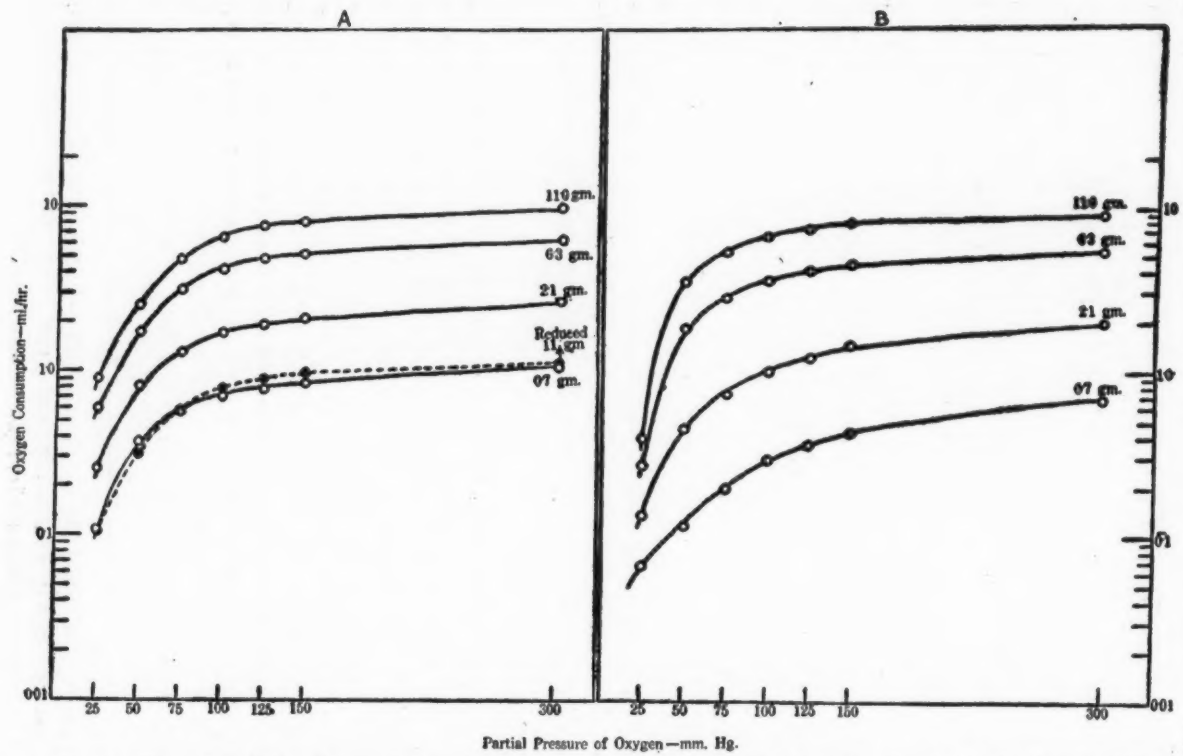

FIG. 4. Illustration of size and dependent respiration at various partial pressures of oxygen. Panel A is the data at 29° C., which was also the acclimation temperature. Panel B is the data at 25° C. The weights of the fish are given at the upper end of each curve. The broken line in Panel A represents the curve of the 11 gm. fish reduced and drawn over that of the 0·7 gm. fish.

Critical Tension

It will be noted from Fig. 4 that the dependency upon oxygen tension generally becomes very pronounced at nearly 150 mm. Hg pO_2, and at almost air-saturation levels, as the curve below this point shows a steep fall. In two species of Chironomids inhabiting a pool where oxygen tension was low and ranged from 3 mm. Hg to 92 mm. Hg, Walshe (1948) found that the critical tension was 35 to 65 mm. Hg. Maloeuf (1937), on the other hand, found that a mussel showed dependency even at pressures four times that of air-saturation. Thus there appears to be various kinds of dependency upon the oxygen tension of the medium in different animals. In the milk fish as well as in the speckled trout (Job, 1955), the critical tension was normally at air-saturation pressures. It seems, therefore, that the critical tension itself is an indication of the intensity of normal metabolic activity; and because of its wide variation, a definition of the critical tension with reference to its range and magnitude appears to have its drawbacks. According to Hayes *et al.* (1951), the critical tension is that which is half maximal and which may extend from air saturation to almost zero. From a comparison of curves for the 0·7 gm. fish in Panels A and B in Fig. 4, such a definition seems untenable, even within the same species.

Oxygen Consumption and Live-fish Transport

In experiments upon milk fish it was found that a 0·7 gm. fish consumed under routine-activity, about 1·1 ml./hr., and a 11·0 gm. fish about 9·84 ml./hr. at 29° C. This was reduced at 25° C. to 0·69 and 8·91 ml./hr., respectively. At the same time, the data obtained at the asphyxial level of oxygen which, however, did not indicate any ' size-effect ', either at 25° C. or at 29° C., showed that the average value was 1·05 ml./litre, at 29° C. and 0·78 ml./litre, at 25° C. While it is possible to make use of the former set of data in estimating the maximum oxygen requirements of the particular size of fish during transport, it is not possible to make use of the latter for setting the lower limit of oxygen for survival. It has already been shown in the case of goldfish (Fry and Hart, 1948), and for the speckled trout (Graham, 1949 and Job, 1955), that in the zone of respiratory dependence when the oxygen uptake is reduced to a level just equal to the standard rate, the animal has already reached the ' incipient lethal level ' (Fry, 1947). At this level, the animal can just maintain itself and is capable of sustaining only the basic ancillary vital activities. But once the rate of uptake falls below this level, the animal can no longer tolerate oxygen lack and is bound, eventually to succumb; but how soon depends upon its ability to resist asphyxia. For a detailed analysis of the incipient lethal level, and its implications in the study of respiratory metabolism see Shepard (1955).

Therefore, in order to establish the minimum tolerance of a fish in transport, to low oxygen tension the lowest oxygen levels permitting indefinite survival must be determined. It would therefore provide a safe margin if the standard rate of oxygen consumption is measured and made use of in fixing the tolerance level of this factor for fish in transport. Apparently Saha *et al.* (1956) have measured the asphyxial level and recommend 0·5 p.p.m. of oxygen as being sufficient for maintaining major carp fry, about 5·08 cm. long, alive for an indefinite period at 31° C. Owing to difficulties in obtaining measurements of the standard rates in this work, it was not possible to incorporate the available data into a general formula for estimating the requirements of live fish during transport. This was attempted by Viswanathan and Tampi (1952), who also based their calculations on the asphyxial level, or to be more precise, on the resistance-times for this species.

SUMMARY

The oxygen consumption of *Chanos chanos* (milk fish) was measured with fish ranging in weight from 0·7 to 11·0 gm., both at 25° and 29° C. They were earlier acclimated to 29° C. During the experiments the fish were kept in a ' routine state of activity '.

The oxygen consumption was measured under the high oxygen tension of 300 mm. Hg, down to almost asphyxial levels. It was found that the critical tension of oxygen at which uptake of the gas becomes dependent upon the available oxygen, was around air-saturation levels (150 mm. Hg), at the acclimation temperature of 29° C. As a result of a sudden change in temperature (25° C.) however acting as an accessory factor, the critical tension varied considerably when related to size. A 0·7 gm. fish showed dependence even at 300 mm. Hg pO_2.

The size temperature relationship for the routine-active metabolism revealed a proportionate rise in the metabolic rate at the higher temperature (29° C.). But the relative rate of increase is greatest in the smaller fish.

The relative ability of the fish to utilise oxygen under proper acclimation (29° C.) showed that it was the same under all partial pressures of the gas in the medium, irrespective of the size of the fish.

Oxygen consumption is discussed in relation to live fish transport.

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